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VARIATIONS IN THE SIZE, WEIGHT AND HISTOLOGIC STRUCTURE OF THE THYROID GLAND

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This study is based on a large number of thyroid glands. My purpose was to determine the effects of various diseases on the histologic structure of the gland.

MATERIAL AND METHODS OF STUDY

The material used in this study consisted of 725 thyroid glands removed intact at autopsies during the period 1929 to 1934. Over 500 of the glands were removed at consecutive autopsies performed by me; the remainder were secured by members of the department of pathology. In common with each case the complete clinical histories and autopsy reports were reviewed. Seven hundred and eight glands belonged to the white race and 17 to the colored. There were 156 from female and 569 from male patients, who were distributed among the age groups as shown in table 1.

The central trend in weight for all thyroid glands examined is represented in table 4 as the average weight for males and females tabulated separately in age groups. After gross and histologic study the glands were divided into a nodular, or adenomatous, group and a group free from pathologic changes which were considered to represent as normal a type of thyroid gland as could be obtained in working with unselected autopsy material. In this study a few scattered small aggregations of lymphocytes in a thyroid gland are considered a normal finding. Thyroids with moderate or extensive lymphocytic infiltration, suppurative foci, marked fibrosis, depletion of colloid, hyperplasia, colloid cysts, extremely large acini, adenomatous nodules or a neoplasm were considered pathologic.

Of the 725 glands, 457, or 63 per cent, were normal, both grossly and microscopically. In the male, 381 of 569, or 67 per cent, were normal; while in the female, 76 of 156, or 48 per cent, were normal.

The causes of death are listed in table 2.

The group of miscellaneous infections was a varied group in which the causative organisms were not determined, such as acute appendicitis, peritonitis, chronic cholecystitis, chronic pyelonephritis and chronic cystitis. The majority of these conditions were probably caused by streptococci, staphylococci or colon bacilli, but no bacteriologic reports were available. Only severe bronchopneumonia, such as occurred secondary to fractures, was tabulated. In each of the 43 cases in which death was due to accidents it occurred within thirty-six hours after the time of injury. In all the cases of syphilis the disease was of the acquired type; in 22 it was tertiary, and in 1 case it was in an early secondary stage. Of

the 7 cases of extrapulmonary tuberculosis, 3 were of miliary tuberculosis; 1 was a case of tuberculosis of the rectum, 1 of tuberculosis of the spine, and 2 were instances of severe, extensive bilateral tuberculosis of the adrenal glands.

The material can be readily combined into major groups as shown in table 3.

TABLE 1.—*Distribution of Patients in Age Groups*

Age Group	Males	Females	Total
Stillbirth	6	4	10
1-30 days	8	4	12
1-12 months	8	5	13
1- 5 years	1	6	7
5-10 years	7	3	10
10-15 years	3	5	8
15-20 years	6	11	17
20-30 years	18	13	31
30-40 years	132	20	152
40-50 years	155	26	181
50-60 years	86	22	108
60-70 years	79	20	99
70-80 years	49	10	59
80-90 years	11	7	18
Total	569	156	725

TABLE 2.—*Causes of Death in the Cases Studied*

Disease	Cases	Disease	Cases
Lobar pneumonia	33	Portal cirrhosis	17
Bronchopneumonia	12	Old rheumatic valve defects	32
Ulcerative colitis	4	Coronary sclerosis	15
Acute streptococcic infections	32	Primary hypertension	73
Diphtheria	4	Cerebral arteriosclerosis	7
Gas gangrene	2	Pulmonary embolism	9
Scarlet fever	3	Chronic glomerulonephritis	19
Tetanus	1	Pernicious anemia	8
Fungous infections	2	Diffuse hyperplastic goiter	2
Miscellaneous infections	109	Accidental injuries	43
Syphilis	23	Chronic alcoholism	6
Pulmonary tuberculosis, chronic	96	Pregnancy (cesarean section)	1
Extrapulmonary tuberculosis	7	Arsphenamine encephalitis	1
Malignant tumors	110	Congenital defects	20
Leukemia	9	Cause of death undetermined	3
Diabetes mellitus	16	Miscellaneous illnesses	6
		Total	725

TABLE 3.—*Causes of Death Combined into Major Groups*

Group	Cases	Group	Cases
Acute infectious diseases	202	Portal cirrhosis	17
Syphilis	23	Circulatory diseases	136
Tuberculosis	103	Diffuse hyperplastic goiter	2
Malignant tumors	119	Accidental injuries	43
Diabetes mellitus	16	Miscellaneous illnesses	73
		Total	725

It may be noted from the foregoing tables that 328 patients died of acute and chronic infectious diseases and that 43 died from accidental injuries.

Special attention was given to the following features: diffuse hyperplasia, focal hyperplasia, depletion or absence of colloid, areas of lymphocytic infiltration, areas of suppurative inflammation, areas of fibrosis and adenomas.

The acini were measured and a diameter of 1 mm. was arbitrarily set as the upper limit of normal.

The colloid content of the acini, as well as the presence of vacuoles in the colloid, was tabulated. The vacuoles have been considered as artefacts or the result of postmortem changes by many workers; however, my work does not support this conception. The tissues were all prepared by the same technic, and the material secured shortly after death was fixed in a 3.7 per cent aqueous solution of formaldehyde. Slides from several cases were even carried at the same time in the same slide-carrier so that all steps in the technic were identical, yet it was observed that one thyroid would be almost entirely free from vacuoles, another would contain a few, and still another, a large number. Depletion and vacuolation of colloid were rather common in persons dying of infectious diseases, while such changes did not occur in the group whose deaths were due to accidents. Vacuolation and depletion of colloid were most numerous and extensive in a case of exophthalmic goiter in which no iodine was administered. The marked depletion of colloid which may occur in acute infections is illustrated by figure 1 *A*.

OBSERVATIONS

Weight of the Thyroid.—The weight of the thyroid at birth is subject to considerable variation, depending largely on the locality. Castaldi [1] reported 1.1 Gm. at Florenz; Guiart,[2] 2.5 Gm. at Paris; Eggenberger,[3] from 1.5 to 3 Gm. at Rome; Wegelin,[4] 1.9 Gm. at Kiel and 8.2 Gm. at Bern; Gloor,[5] 4.5 Gm. at Geneva; Klöppel,[6] 4.7 Gm. at Göttingen, 5.7 Gm. at Berlin and 10.5 Gm. at Freiburg. Rice [7] reported 1.39 Gm. for stillborn infants and 1.78 Gm. for infants from 1 to 10 days old, based on his study of a series of thyroids from Minnesota. Lucien and George [8] give the relative weight, i. e., the relation of thyroid weight to body weight, at birth as from 1:1,400 to 1:1,700, while Thomas [9] calculated the relative weight at birth as varying between 1:400 and 1:243. Klöppel observed that the weight of the thyroid decreased after birth and often remained less during the first year. Wegelin gave the weight at birth as 1.9 Gm., at 6 months as 1.55 Gm. and at 1 year as 2.40 Gm. The average weight of the normal thyroid in the male stillborn infant was 2.1 Gm. and in the female 1.6 Gm.; at from 1 to 30 days of age the normal thyroid in the male weighed 1.9 Gm. and that in the female 2.1 Gm. (table 4).

1. Castaldi, L.: Arch. ital. di anat. e di embriol. **18**:97, 1922; cited by Wegelin,[4] p. 15.

2. Guiart, J., cited by Wegelin,[4] p. 15.

3. Eggenberger, in Hirsch, M.: Handbuch der inneren Sekretion, Leipzig, Curt Kabitzsch, 1928; cited by Wegelin,[4] p. 15.

4. Wegelin, C., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 8, p. 16.

5. Gloor, H. U.: Frankfurt. Ztschr. f. Path. **34**:504, 1923.

6. Klöppel, F. C.: Beitr. z. path. Anat. u. z. allg. Path. **49**:579, 1910.

7. Rice, C. O.: West. J. Surg. **39**:931, 1931.

8. Lucien, M., and George, A., cited by Wegelin,[4] p. 15.

9. Thomas, E.: Innere Sekretion in der ersten Lebenszeit (vor und nach der Geburt), Jena, Gustav Fischer, 1926, p. 134.

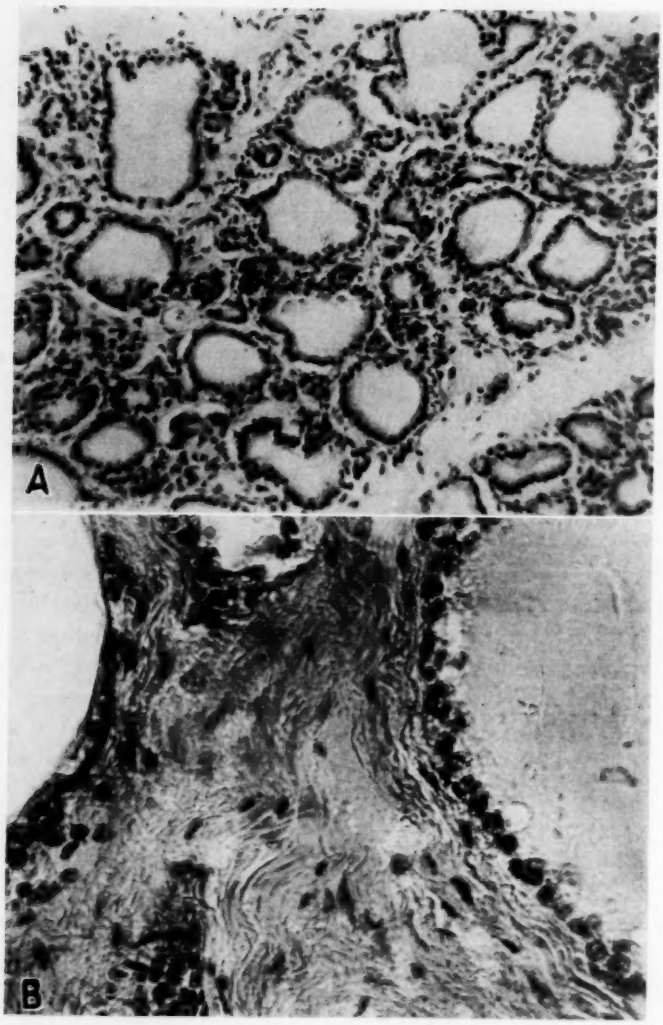


Fig. 1.—*A*, marked depletion of colloid in a thyroid gland from a boy 8 years of age, who died of acute bacillary dysentery. Note that the follicles are almost devoid of colloid. *B*, area of fibrosis in a thyroid gland. Note that the space between the follicles is much wider than normal and that the loose reticular connective tissue has been replaced by dense fibrosis and collagenous bands.

The weight of the adult thyroid gland, according to Gray's Anatomy,[10] is somewhat variable; usually it is about 30 Gm. Marine [11] stated that the normal thyroid weighs between 20 and 25 Gm., does not exceed 0.35 Gm. per kilogram of body weight and is relatively larger in women and much larger in infants. In the Chicago region, Jaffé [12] found the average weight in white men to be 27.4 Gm. and that in white women 28.1 Gm. Boyd observed that the weight varied with the geographic position and was about a third heavier in the female. He gave the average weight at the seacoast as between 20 and 30 Gm., and that in hilly districts as between 35 and 50 Gm.

TABLE 4.—*Incidence and Average Weight of the Normal Thyroid Gland for Males and Females in Age Groups*

	Males					Females				
	Thyroids in Entire Series		Normal Thyroids			Thyroids in Entire Series		Normal Thyroids		
Age Groups	Total Number	Average Weight, Gm.	Number	Percentage	Average Weight, Gm.	Total Number	Average Weight, Gm.	Number	Percentage	Average Weight, Gm.
Stillbirths	6	2.1	6	..	2.1	4	1.6	4	..	1.6
1-30 days	8	1.9	8	..	1.9	4	2.1	4	..	2.1
1-12 mos.	8	2.4	8	..	2.4	5	3.3	5	..	3.3
1- 5 years	1	7.5	1	..	7.5	6	3.6	2	..	4.3
5-10 years	7	9.5	1	..	12.0	3	6.2	2	..	6.3
10-15 years	3	16.8	2	..	21.5	5	22.2	5	..	22.2
15-20 years	6	23.3	5	..	25.5	11	29.7	9	..	25.1
20-30 years	18	31.0	11	61	28.0	13	27.2	9	69	17.7
30-40 years	132	27.7	114	86	24.9	20	34.2	7	35	31.0
40-50 years	155	28.1	122	79	24.1	26	32.0	12	46	23.5
50-60 years	86	31.6	41	48	25.9	22	36.7	8	36	22.0
60-70 years	79	32.0	42	53	24.0	20	34.6	5	25	19.6
70-80 years	49	35.6	17	36	22.0	10	46.0	2	20	19.5
80-90 years	11	28.2	3	27	20.1	7	38.0	2	28½	39.5
Total	569		381	67		156		76	48	

The incidence and average weight of the normal thyroid gland for males and females in each age group are given in table 4 and compared with the corresponding age distribution and weight of all the thyroid glands collected in this investigation.

From inspection of the scatter diagrams, the cases in the third to the ninth decade in males and the third to the eighth decade in females were selected as the most suitable for statistical analysis.

The distribution of the weight of the normal thyroid gland for the male and the female as plotted on the age at yearly intervals is shown in figure 2.

10. Lewis, W. H.: Anatomy of the Human Body, Philadelphia, Lea & Febiger, 1930, p. 1262.

11. Marine, D.: Special Cytology, New York, Paul B. Hoeber, Inc., 1932, p. 803.

12. Jaffé, R. H.: Arch. Path. **10**:887, 1930.

Both the median and the average weight of the normal thyroid gland of the male are presented in table 5 for the third to the ninth decade. The average weight is the greatest at 28 Gm., at the median age of 26 years in the third decade, drops to 24.9 Gm. in the fourth decade, rises slightly in the sixth decade, to 25.9 Gm., and reaches the lowest value, 22 Gm., in the eighth decade. These figures are paralleled fairly well by the median weights. It will be noted that there is considerable range of weight in the normal glands, the lightest weighing 11 Gm. in the seventh decade and the heaviest 57 Gm. in the fourth decade.

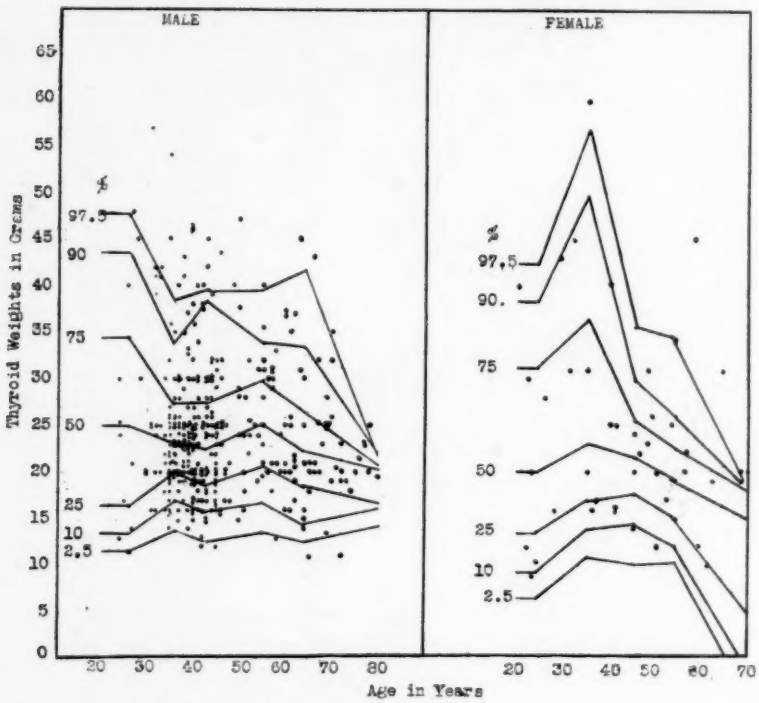


Fig. 2.—Percentage zones of normal variation in the weight of the adult thyroid gland.

Percentage expected	2.5	7.5	15	25	25	15	7.5	2.5
Males: 347								
Percentage observed	1.7	7.5	14	24.8	24.5	16	6	5.5
Females: 41								
Percentage observed	.0	7.3	17	17	27	17	7.3	7.3

The median and the average weight of the thyroid gland of the female in the third to the eighth decade are recorded in table 6. Since there were only 2 cases in the eighth decade, these were not included in the analysis.

The average weight and also the median weight of 31 Gm. occurs at the median age of 35 years in the fourth decade. The average weight is 17.7 and the median weight 20 Gm. in the third decade. Following the fourth decade there is a progressive drop in weight with each decade until a median weight of 19 and an average weight of 19.6 Gm. are reached in the seventh decade.

The percentile method was utilized in an effort to establish the probable limits of normal variation in the thyroid gland in the male for the third to the ninth decade and in the female for the third to the eighth decade. According to the method described by Boyd,[13] cumu-

TABLE 5.—*Weight of the Normal Thyroid Gland in the Male in the Third to the Ninth Decade*

Age Interval, Years	Patients	Median Age, Years	Median Weight of Thyroids, Gm.	Average Weight, Gm.	Weight Range, Gm.	Total Weight Range, Gm.
20-30	11	26	26.5	28.0	13 through 48	35
30-40	114	36	30.0	24.9	14 through 57	43
40-50	122	43	24.0	24.1	12 through 42	30
50-60	41	55	25.0	25.9	13 through 47	34
60-70	42	64	21.0	24.0	11 through 45	34
70-80	17	72	20.5	22.0	12 through 35	23

TABLE 6.—*Weight of the Normal Thyroid Gland in the Female in the Third to the Eighth Decade*

Age Interval, Years	Patients	Median Age, Years	Median Weight of Thyroids, Gm.	Average Weight, Gm.	Weight Range, Gm.	Total Weight Range, Gm.
20-30	9	24	20.0	17.7	8 through 43	35
30-40	7	35	31.0	31.0	16 through 60	44
40-50	12	45	23.0	23.5	14 through 40	26
50-60	8	54	20.0	22.0	12 through 45	33
60-70	5	64	19.0	19.6	10 through 31	21

lative frequency curves were constructed separately for males and females for each of the decades named. The percentiles 97.5, 90, 75, 50, 25, 10 and 2.5 for each decade were plotted at the median age and the corresponding percentiles joined by continuous lines. The weight of the thyroid gland in grams for the age in yearly intervals was then plotted as is shown in figure 2.

Conception of Adenomatous Goiter.—Nodose goiters were found by Wegelin in 73.3 per cent of men over 20 years old and in 88.4 per cent of women over this age. Clerc[14] in Bern observed nodules in practically all thyroids removed from persons over 50 years of age, while

13. Boyd, Edith: Anat. Rec. **62**:3 (April 25) 1935.

14. Clerc: Frankfurt. Ztschr. f. Path. **10**:1, 1912.

Klöppel in Freiburg gave a figure of 81 per cent for people over 50 years old. In the region of Chicago, Jaffé reported 30 per cent nodose thyroids in males and 44.7 per cent in females. In Minnesota, Rice found nodules in 43.8 per cent of males and 53.1 per cent of females. The incidence and average weight of nodular glands are recorded in table 7. Nodules were found in 191 of the 725 glands, or 26.3 per cent. There were 67 nodular thyroids among the 156 glands from females, or 42 per cent, and 124 nodular thyroids among the 569 glands from males, or 22 per cent. The incidence of nodules in females is roughly twice that in males. Nodular thyroids are encountered more frequently with increase of age in both sexes, while they are relatively uncommon below 15 years of age.

TABLE 7.—*Incidence and Average Weight of the Nodular Thyroid Gland for Males and Females in Age Groups*

	Males					Females				
	Thyroids in Entire Series		Nodular Glands			Thyroids in Entire Series		Nodular Glands		
Age Groups	Total Number	Average Weight, Gm.	Number	Average Weight, Gm.	Percentage	Total Number	Average Weight, Gm.	Number	Average Weight, Gm.	Percentage
Stillbirths	6	2.1	....	....	..	4	1.6	..	....	..
1-30 days	8	1.9	....	....	..	4	2.9	..	....	..
1-12 mos.	8	2.4	..	....	..	5	3.3	..	....	..
1- 5 years	1	7.5	....	....	..	6	3.6	1	2.5	..
5-10 years	7	9.5	....	....	..	3	6.2	0	....	..
10-15 years	3	16.8	....	....	..	5	22.2	0	....	..
15-20 years	6	23.3	1	14.0	17	11	29.7	2	50.2	18
20-30 years	18	31.0	4	39.5	22	13	27.2	2	18.2	15
30-40 years	132	27.7	12	45.0	9	20	34.2	11	41.5	55
40-50 years	155	28.1	21	39.5	13½	26	32.0	12	35.8	46
50-60 years	86	31.6	29	33.5	34	22	36.7	13	46.5	59
60-70 years	79	32.0	28	43.5	35½	20	34.6	15	41.4	75
70-80 years	49	35.6	26	45.9	53	10	46.0	6	58.5	60
80-90 years	11	28.2	3	27.0	27	7	38.0	5	48.0	91½
Total	569		124		22	156		67		42

The average weight of the nodular thyroids is considerably more than that of the normal in all age groups for both sexes. The greatest average weight is that found for males and females in the eighth decade.

The weights of the nodular thyroid glands for males and females are plotted on the age at yearly intervals in figure 3.

In the males the observed data fit fairly well the percentages expected with a slight weighing past the 97.5 percentile. The number of thyroids from females is somewhat small, but the percentile fits surprisingly well the amount of data observed as compared with the expected percentage. In the series from males 49.3 per cent lie between the 25 and 75 percentiles, and in that from females 44 per cent are between the same limits. In the series from males 92.8 are included in the 2.5 to 97.5 percentiles and in that from the female 92.7 per cent. In

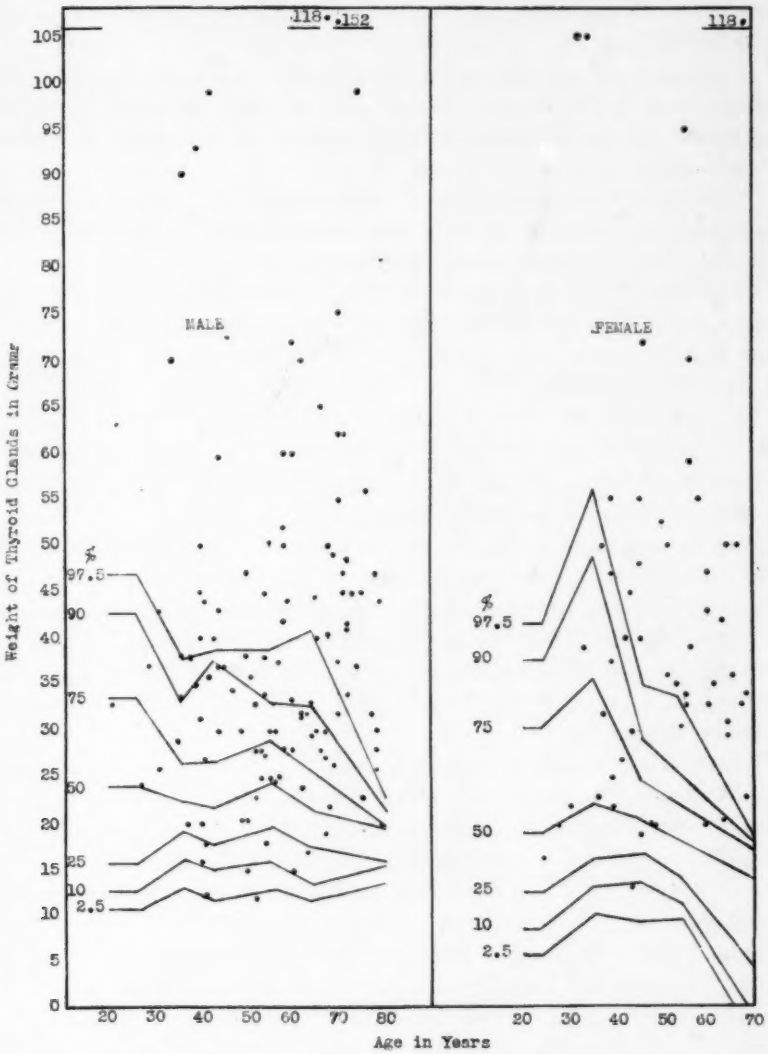


Fig. 3.—Scatter diagram of weights of nodular thyroid glands from males and females, plotted over the percentile zones of normal variation.

Percentage expected	2.5	7.5	15.0	25	25	15.0	7.5	2.5
Males: 120								
Percentage observed	1.0	1.0	3.5	5	10	23.5	10.	45.0
Females: 53								
Percentage observed	0.	2.0	0.0	6	17	8.0	8.0	59.0

the male the trend is downward from the third decade and in the female from the midpart of the fourth decade. The weight trend of the thyroid gland drops in both sexes after middle life but to a somewhat greater extent in the female.

In figure 3 the weights of the nodular thyroid glands for males and females have been plotted by the same method over the percentile determined for the corresponding age groups in the series of normal thyroid glands.

With the aforementioned percentile method it is evident that the weights of the nodular thyroids tend toward much higher values for both males and females than the normal glands.

Only 15 per cent of the thyroids from males and 23 per cent of those from females lie between the 25 and 75 percentiles and 54 per cent of those from males and 41 per cent of those from females between the 2.5 and 97.5 percentiles.

The occurrence of diffuse areas of fibrosis is presented in table 8. This condition was found in 71, or 9.7 per cent, of the 725 thyroids. The number of cases in each disease group is too small for a percentage calculation to be of particular significance; however, one notes that fibrosis is apparently less common in those who die of infectious diseases aside from syphilis than in those whose deaths were due to accidental injuries and twice as high in those who had circulatory diseases. An analysis of the data reveals that the circulatory diseases fall in the older age groups, thus leading to the conclusion that fibrosis is a concomitant of advancing age rather than of disease, with the possible exception of syphilis.

There was decrease of colloid in 89, or 12.3 per cent, of all cases. The incidence in relation to various disease conditions is analyzed in table 9. No evidence of depletion of colloid, vacuolation of colloid or decrease in acidophilic staining was observed in the glands from 43 persons whose death was due to accidental causes. Partial depletion of colloid was rather common in patients with acute infectious diseases and also high in those with diabetes mellitus, although in the latter the number of cases was insufficient to be of any great significance.

Focal hyperplasia was observed in 9 thyroids and diffuse hyperplasia in 2, as shown in table 10. In none of the glands from persons who suffered accidental death was there any hyperplasia, while in the 2 representing persons with exophthalmic goiter it was most severe. Hyperplasia of the focal type occurred 5 times in association with infectious diseases, once with tuberculosis and 3 times with malignant tumors.

Twenty thyroid glands, or 2.7 per cent of the entire series, contained focal areas of suppurative inflammation. The areas ranged from scattered collections of polymorphonuclear neutrophilic leukocytes to very large, extensive foci which somewhat suggested acute thyroiditis.

TABLE 8.—*Thyroids Containing Diffuse Areas of Fibrosis*

Disease Group	Thyroids in Entire Series	Glands Showing Fibrosis	Percentage
Miscellaneous infectious diseases	202	15	7.4
Syphilis	23	4	17.3
Tuberculosis	103	7	6.7
Malignant tumors	110	10	9.0
Diabetes mellitus	16	2	12.6
Portal cirrhosis	17	1	5.8
Circulatory diseases	136	25	18.3
Diffuse hyperplastic goiter	2	1	50.0
Accidental deaths	43	4	9.3
Other groups	73	2	2.9
Total	725	71	9.7

TABLE 9.—*Depletion of Colloid*

Disease Group	Thyroids in Entire Series	Glands Showing Depletion	Percentage
Lobar pneumonia	33	7	
Bronchopneumonia	12	10	
Ulcerative colitis	4	3	
Acute streptococcic infections	32	7	
Diphtheria	4	4	
Gas gangrene	2	1	
Scarlet fever	3	1	
Tetanus	1	0	
Fungous infections	2	1	
Miscellaneous infections	109	10	
Total for infections	202	34	16.8
Syphilis	23	2	
Tuberculosis	103	11	10.6
Malignant tumors	110	14	12.7
Leukemia	9	0	
Diabetes mellitus	16	5	
Portal cirrhosis	17	2	
Old rheumatic valve defects	32	3	
Coronary sclerosis	15	2	
Hypertension	73	10	
Cerebral arteriosclerosis	7	0	
Pulmonary embolism	9	1	
Total for circulatory diseases	136	16	11.7
Diffuse hyperplastic goiter	2	2	
Miscellaneous deaths	64	3	
Accidental deaths	43	0	
Grand total	725	89	12.3

TABLE 10.—*Focal Hyperplasia in the Thyroid*

Disease Group	Thyroids in Entire Series	Glands Showing Hyperplasia	Percentage
Miscellaneous infectious diseases	202	5	2.4
Syphilis	23	0	...
Tuberculosis	103	1	0.9
Malignant tumors	110	3	2.7
Diffuse hyperplastic goiter	2	2	...
Accidental deaths	43	0	...
Miscellaneous groups	242	0	...
Total	725	11	1.5

The distribution of these areas in relation to the various diseases is given in table 11. This condition appears to be somewhat more common in association with acute infections, rheumatic defects of the cardiac valve and coronary sclerosis.

TABLE 11.—*Thyroids Containing Focal Areas of Suppurative Inflammation*

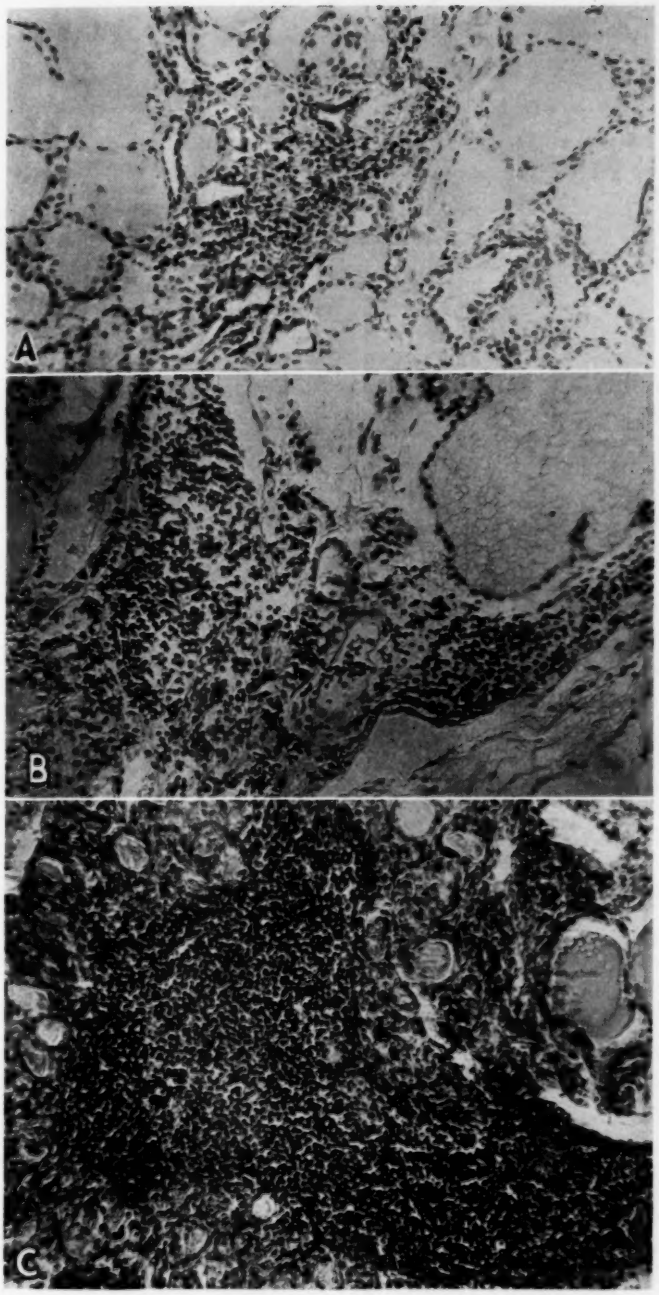
Disease Group	Thyroids in Entire Series	Glands Showing Focal Suppuration	Percentage
Other infections	152	0	
Acute streptococcic infections	32	2	
Tetanus	1	1	
Duodenal ulcer	5	1	
Bronchopneumonia	12	2	
Total for group infections	202	5	2.4
Syphilis	23	1	
Tuberculosis	103	1	0.9
Malignant tumors	110	3	2.7
Hypertension	73	1	
Coronary sclerosis	15	2	
Cerebral arteriosclerosis	7	0	
Old rheumatic valve defects	32	3	
Pulmonary embolism	9	0	
Total for circulatory diseases	136	6	4.4
Diabetes mellitus	16	1	
Accidental death	43	1	2.3
Arsphenamine encephalitis	1	1	
Miscellaneous groups	91	0	
Grand total	725	20	2.7

TABLE 12.—*Incidence of Grades I, II and III of Lymphocytic Foci*

Age Group	In Males				In Females				Combined Total
	I	II	III	Total	I	II	III	Total	
1-30 days	..	1	..	1	..	..	..	..	1
1- 5 years	..	..	..	..	..	1	..	1	1
10-20 years	..	..	..	..	..	..	..	..	...
20-30 years	3	1	..	4	3	..	..	3	7
30-40 years	19	5	..	24	3	1	1	5	29
40-50 years	16	2	2	20	7	1	..	8	28
50-60 years	12	5	..	17	4	4	..	8	25
60-70 years	10	5	1	16	4	3	3	7	23
70-80 years	7	2	..	9	2	..	1	3	12
80-90 years	..	1	..	1	3	..	..	3	4
Total	70	22	3	95	27	10	2	39	134

Lymphocytic foci were encountered in 134 thyroids, or 18.4 per cent. of the series. The foci were graded as I, II and III according to their size and extent (fig. 4) and tabulated according to age groups (table 12).

Lymphocytic foci were of more frequent occurrence in females than in males; 20 per cent of the glands showing such foci were from males, whereas 29 per cent were from females. The incidence of lymphocytic foci in the various age groups is shown in table 13.



EXPLANATION OF FIGURE 4

A, lymphocytic foci, grade I. Note the distribution of the lymphocytes in the interfollicular stroma.

B, lymphocytic foci in the thyroid, grade II. Note the large collections of lymphocytes in the interfollicular stroma and about the blood vessels.

C, lymphocytic foci, grade III. Note the large numbers of closely packed lymphocytes encroaching on, and infiltrating between, the surrounding follicles.

TABLE 13.—*Incidence of Lymphocytic Foci*

Age Group	Thyroids	Glands Showing Lymphocytic Foci	Percentage
5-10 years	10	4	
10-20 years	24	0	
20-30 years	33	7	21.2
30-40 years	186	29	15.6
40-50 years	160	28	17.5
50-60 years	107	25	23.3
60-70 years	99	23	23.3
70-80 years	49	12	24.5
80-90 years	12	4	

TABLE 14.—*Lymphocytic Foci in the Thyroid*

Disease Group	Thyroids in Entire Series	Glands Showing Foci	Percentage
Lobar pneumonia	33	7	
Bronchopneumonia	12	4	
Ulcerative colitis	4	2	
Acute streptococcic infections	32	3	
Diphtheria	4	2	
Gas gangrene (Bacillus Welchii)	2	1	
Scarlet fever	5	1	
Tetanus	1	1	
Fungous infections	2	0	
Miscellaneous infections	109	16	
Total for infections	202	37	18.3
Syphilis	23	5	21.7
Tuberculosis			
Pulmonary	96	10	10.4
Extrapulmonary	7	..	
Total for tuberculosis	103	10	9.7
Malignant tumors	110	25	22.7
Leukemia	9	0	
Diabetes mellitus	16	5	
Portal cirrhosis	17	4	
Old rheumatic valve defects	32	8	25.0
Coronary sclerosis	15	5	
Hypertension, primary	73	18	24.6
Cerebral arteriosclerosis	7	0	
Pulmonary embolism	9	0	
Total for circulatory diseases	136	31	22.7
Chronic glomerulonephritis	19	3	
Pernicious anemia	8	2	
Diffuse hyperplastic goiter	2	2	
Accidental deaths	43	6	13.9
Chronic alcoholism	6	1	
Pregnancy	1	1	
Arsphenamine encephalitis	1	1	
Congenital defects	20	0	
Cause of death undetermined	3	1	
Miscellaneous	6	0	
Grand total	725	134	18.4

There were 6 thyroids with grade I lymphocytic foci from the 43 persons who died as a result of accidents. Such foci are of rather frequent occurrence in association with acute and chronic infections, malignant tumors, circulatory diseases, diabetes mellitus and pernicious anemia, so that one must conclude that there is apparently no direct relation between this phenomenon and the diseases listed in table 14, aside from diffuse hyperplastic goiter.

Intense, acute hyperemia of the thyroid was noted in a 6 month old boy dying of encephalitis with terminal bronchopneumonia. The capillaries were distended throughout the stroma and filled with erythrocytes. Intense capillary congestion was also observed in the thyroid of a 26 year old man suffering from purulent meningitis arising from suppurative frontal sinusitis. It was noted to a lesser extent in thyroids from patients with other acute infectious diseases but was not a constant finding. While there was occasional desquamation of a few cells, extensive desquamation of large quantities of epithelium into the follicles was not observed. Diffuse colloid in the interacinar spaces was sometimes noted associated with conditions in which it was somewhat depleted in the follicles. Medial calcification in the larger arteries was found in a few thyroids belonging to the older age groups.

There were 3 persons with malignant melanoma, and in 2 of these the thyroid contained multiple metastatic nodules. These nodules measured from 2 to 3 mm. in diameter and on macroscopic examination could be made out as poorly defined blackish areas. There was one person with primary carcinoma of the thyroid of an undifferentiated type, with metastasis to the regional lymph nodes, lungs and liver. The tumor was primary in the right lobe, with no involvement of the left. The left lobe was entirely normal, showing no evidence of colloid depletion, hyperplasia or lymphocytic or exudative infiltration.

SUMMARY AND CONCLUSIONS

A series of 725 thyroids obtained at autopsies from 569 males and 156 females has been investigated, both grossly and microscopically. Sixty-three per cent of all the glands were normal: 67 per cent of the glands from males and 48 per cent of those from females. As age increased, the incidence of normal glands was less, this difference being more marked in the females.

The average weight of the normal thyroids in adult males between 20 and 80 years varied between 28 and 22 Gm. The percentile zones of variation in the normal thyroid gland form the most reliable index of weight trends.

Nodules occurred in 191, or 26.3 per cent, of the material, 42 per cent of the glands in the female group and 22 per cent of those in the

male group, the incidence in females being roughly twice that in males. Nodular thyroids were relatively uncommon in persons less than 15 years old, and the incidence increased with age. The average weight of nodular thyroids was considerably more than the normal in all age groups for both sexes.

Areas of diffuse fibrosis were found in 71 thyroids, or 9.7 per cent. Fibrosis is relatively more common with increase in age.

Depletion of colloid was more common with acute infectious disease and did not occur in 43 persons whose deaths were accidental.

Areas of focal hyperplasia were observed in 9 thyroids and diffuse hyperplasia in 2.

Twenty glands, or 2.7 per cent of the material, contained areas of suppurative inflammation, which were somewhat more commonly associated with acute infections, rheumatic defects of the cardiac valves and coronary sclerosis.

Lymphocytic foci were found in 134 thyroids, or 18.4 per cent of the material, and were somewhat more common in females. The lymphocytic foci were constant at 23 per cent in the fifth, sixth, seventh and eighth decades and highest in the 5 to 10 year old group, at 40 per cent. Apparently there is no definite relation between the presence of lymphocytic foci and different types of disease, apart from exophthalmic goiter

THE CHEMICAL REACTION BETWEEN OLEIC ACID AND AQUEOUS SOLUTIONS OF MAGNESIUM

ITS PATHOLOGIC SIGNIFICANCE

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The evolution of many vital activities in tissues has been clarified through studies demonstrating fundamental chemical reactions between the substances involved. The wide distribution of fats in the body has been established by chemical extraction and quantitative fractionation, but a larger volume of information, especially with reference to disease, has been derived by qualitative stains for fats in histologic preparations of tissues. The insolubility of fats in aqueous solutions of the tissue fluids has stood as a barrier to understanding how at water-oil interphases chemical reactions can take place between the two mediums. Chemical reactions between fatty acids, the products of hydrolysis of fat, and aqueous solutions of various bases have not been studied thoroughly. As suggested in a previous report [1] the fatty acids appear to bridge this gap between fats and aqueous mediums, so that chemical reactions take place between the two systems. Klotz [2] embedded capsules filled with fat and fatty acids in animal tissues. After several days the fat material contained calcium soap, and the calcium content was much greater than that of normal blood and lymph. Langmuir and Schaefer [3] recently found that monomolecular films of stearic acid floating on water containing calcium or barium salts were converted completely or in part into the corresponding soaps of the fatty acids and that the extent of the reaction depended on the concentration of hydrogen ions in the aqueous solution.

The chemical reactions between sodium hydroxide or potassium hydroxide and a fatty acid such as oleic acid are well known. In the presence of a considerable excess of oleic acid the sodium soap dis-

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From the Henry Baird Favill Laboratory of St. Luke's Hospital and the Department of Chemistry of the University of Chicago.

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2. Klotz, O.: J. Exper. Med. **7**:633, 1905; **8**:322, 1906.
3. Langmuir, I., and Schaefer, V. J.: J. Am. Chem. Soc. **58**:284, 1936.

tributes itself between the aqueous and the oil phase. With calcium hydroxide and an excess of oleic acid the relations are different. Since the calcium oleate which is formed is extremely insoluble in water and readily soluble in oleic acid, the calcium oleate at equilibrium is found only in the oil phase.

The fluids in animal tissues do not contain free bases such as sodium hydroxide or calcium hydroxide. Therefore, the reaction between fatty acids and aqueous salt solutions was investigated. Oleic acid and dilute aqueous solutions of magnesium acid phosphate ($MgHPO_4$) were chosen for the initial experiments. The reaction between these substances is given by equation 1, or ionically, by equation 2.

$$\underset{(Aq.)}{MgHPO_4} + 2\underset{(oil)}{C_{17}H_{33}COOH} \rightleftarrows \underset{(oil)}{Mg(C_{17}H_{33}COO)_2} + \underset{(Aq.)}{Mg(H_2PO_4)_2} \quad (1)$$

$$\underset{(Aq.)}{Mg^{++}} + 2\underset{(oil)}{C_{17}H_{33}COOH} \rightleftarrows \underset{(oil)}{Mg(C_{17}H_{33}COO)_2} + 2\underset{(Aq.)}{H^+} \quad (2)$$

The acid phosphate ions buffer the solution, preventing rapid accumulation of free hydrogen ions. This report is a study of the equilibrium represented in equations 1 and 2 and the extent to which this equilibrium is displaced by changes in the different variables involved.

APPARATUS FOR p_H MEASUREMENTS

The p_H measurements were made with the modified spiral type glass electrode and vacuum tube potentiometer described elsewhere.[4] The true electromotive force of all samples was calculated by method 2 of that article. The spiral type glass electrode cell was calibrated daily with phosphate buffers made according to Hastings and Sendroy.[5] The construction of the cell was modified slightly so that the solution to be measured was forced by nitrogen under pressure from the storage bottle or flask into the top of the spiral. This modification avoided contamination of the solution with the buffer solution at the liquid junction. The samples were discarded through the side arm of the stopcock at the bottom of the spiral.

MATERIALS

The oleic acid was prepared from fresh beef fat. In a 500 cc. Erlenmeyer flask 100 Gm. of fat was mixed with 100 cc. of alcohol and a solution of 20 Gm. of sodium hydroxide in 50 cc. of water. The mixture was saponified on a water bath. The warm solution was poured into a large flask containing 4 liters of distilled water and 60 cc. of concentrated hydrochloric acid. The mixture was heated on a water bath until a clear upper oil layer of mixed fatty acids was obtained. The two layers then were separated, and the fatty acids were washed with distilled water until all of the mineral acid had been removed. The saturated acids in the mixed fatty acids were separated by the Twitchell method.[6] (Lead salts of saturated acids are insoluble, and lead salts of unsaturated acids are soluble in

4. Hartsuch, P. J.: J. Infect. Dis. **59**:183, 1936.

5. Hastings, A. B., and Sendroy, J., Jr.: J. Biol. Chem. **61**:695, 1924. Clark, W. M.: The Determination of Hydrogen Ions, ed. 3, Baltimore, Williams & Wilkins Company, 1928, p. 212.

6. Twitchell, E.: Indust. & Engin. Chem. **13**:806, 1921.

95 per cent alcohol at 15 C.) The alcoholic filtrate containing the oleic acid with some lead oleate was evaporated to about one fourth of its original volume on a water bath at 70 C. The concentrated filtrate was poured into 4 liters of distilled water containing about 30 cc. of concentrated hydrochloric acid and the mixture heated for several hours on a water bath at 70 C. to decompose the lead soap. The liberated oleic acid was agitated further with one or two smaller portions of dilute hydrochloric acid solution until the wash water gave no test for lead. The oleic acid was then washed with distilled water in a separatory funnel until a negative test for chloride ions was obtained. The oleic acid was separated from the water as completely as possible, chilled to zero centigrade to break up emulsified water, then filtered through a dry quantitative filter paper.

Samples of oleic acid obtained by this method were nearly colorless (a very light yellow) and odorless and had iodine numbers ranging from 88 to 91 (90, theoretical), acid numbers from 197 to 201 (199, theoretical) and final melting points from 6.6 to 7.5 C., with a spread of from 2 to 2.3 C. between incipient and complete melting.

A magnesium phosphate precipitate of a composition approximating $MgHPO_4$ was formed when solutions containing equivalent amounts of Mallinckrodt ("analytical reagent quality") $MgCl_2.6H_2O$ (or $MgSO_4.7H_2O$) and $Na_2HPO_4.12H_2O$ were mixed. The precipitate was agitated with several portions of water until the wash water gave a negative result in a test for chloride ions (Cl^-) or sulfate ions (SO_4^{--}). Aqueous solutions of magnesium phosphate ($MgHPO_4$) were made by the saturation, with this precipitate, either of redistilled water or of redistilled water to which a known amount of phosphoric acid had been added. The p_H of the solution could be controlled within limits by the amount of phosphoric acid used.

The small percentage of oxygen in the commercial water-pumped tank nitrogen was removed by passage of the gas through a pyrex tube five-eighths inch (1.6 cm.) in diameter which was filled with copper turnings and heated to 475 C. The turnings had been oxidized with air and reduced with hydrogen gas several times to provide an active surface. Frequent tests of the purified gas for traces of oxygen with the apparatus designed by Mugdan and Sixt [7] indicated that the oxygen content of the gas was 0.02 per cent or less, though the unpurified gas contained from 0.2 to 0.3 per cent oxygen.

EXPERIMENTS

Removal of Water-Soluble Oxidation Products from the Oleic Acid Samples.—All experiments were completed in the absence of oxygen, because oleic acid oxidizes slowly in air or oxygen.[8] Several substances are said to be formed by this oxidation, including organic acids of lower molecular weight, such as nonoic acid and traces of acetic and formic acids, all of which are more soluble in water than is oleic acid. This coincides with the observations of Cruto [9] that aqueous solutions in contact with oleic acid and air become more acid. Such an increase in acidity

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(i. e., lowering of p_H) was undesirable in this work because attainment of equilibrium would be impossible and because the decrease in p_H due to the oxidation of the oleic acid could not be separated from the decrease in p_H due to the reaction expressed in equation 2. To ascertain the extent to which oleic acid could lower the p_H of distilled water in an air atmosphere, 2 cc. samples were layered over 200 cc. portions of distilled water in two 250 cc. pyrex bottles. The p_H of the aqueous phase was measured at intervals, and the volume thus lost was made up with distilled water. The p_H fell steadily during twenty days to between 3.75 and 3.77 with no noticeable diminution in the rate of acid formation after twenty days, 50 cc. samples of the two aqueous solutions required 5.20 and 6.25 cc., respectively, of 0.00922 normal sodium hydroxide to neutralize the acids formed; the acid number of the oleic acid over the aqueous solutions had fallen 11.1 points, and the iodine number 14.1 points.

A different behavior was observed when 2 cc. portions of oleic acid were shaken in an atmosphere of nitrogen with eight successive 200 cc. portions of redistilled water which had been boiled and then cooled in an atmosphere of nitrogen. As the small amount of water-soluble impurities was transferred from the oleic acid to the water, the p_H of the aqueous phase, originally 4.5, rose with each new portion to between 5.6 and 5.7. Probably the slight solubility of the oleic acid itself reduced the p_H of distilled water to this value, since distilled water has no buffer capacity. Every sample of oleic acid used subsequently in the experiments with magnesium was agitated in an atmosphere of nitrogen for approximately two hours at room temperature with each of at least ten 200 cc. portions of recently boiled redistilled water.

Technic Employed to Obtain the Heterogeneous Equilibrium.—The following procedure was employed in the mixing of a solution of magnesium acid phosphate ($MgHPO_4$) with oleic acid and in the determination of the extent of the ensuing reaction. Nitrogen was bubbled for several hours through an aqueous solution of magnesium acid phosphate of the desired magnesium concentration and p_H. The solution was then forced under nitrogen pressure into a 250 cc. pyrex bottle equipped with inlet and exit tubes of pyrex, in which the air had previously been replaced by nitrogen. After the solution was agitated in the bottle for an hour, it was forced back under nitrogen pressure into the storage flask. This process was repeated; the third time each bottle was filled to a 200 cc. mark. The average error was ± 1.2 per cent from 200 cc. This rinsing process assured a uniform initial concentration of hydrogen ions in each bottle.

Weighed samples of the washed oleic acid were transferred to the bottles, the stoppers replaced at once, and nitrogen bubbled through the bottles for an hour or two to remove all air from the upper part of each bottle. The contents of the bottles were then agitated gently for from twenty-four to forty-eight hours, after which the p_H of the aqueous phase was determined without admission of air. To accomplish this, samples of the aqueous phase were forced under nitrogen pressure directly into the top of the spiral glass electrode.

After the determination of p_H, the two phases were separated by filtration, and each was analyzed for magnesium content.

(*a*) Analysis of Aqueous Phase: Samples, 50 cc. each, were made acid with 3 cc. of hydrochloric acid diluted 1:3; enough 0.1 molar disodium phosphate (Na_2HPO_4) was added to provide a 50 per cent excess (after allowance for the phosphate already in the solution) and 6.3 cc. of concentrated ammonium hydroxide added dropwise with constant stirring. After this mixture had stood over night the precipitate was filtered onto a weighed Jena glass filtering crucible

with a fritted glass bottom of medium porosity, washed with a 1:9 solution of ammonium hydroxide, then with 95 per cent alcohol and finally with ether, and weighed as $MgNH_4PO_4.6H_2O$, according to Fales' method.[10]

(*b*) Analysis of Oil Phase: The oil was poured from the filter paper into a 125 cc. glass-stoppered Erlenmeyer flask, and 40 cc. of 0.1 molar hydrochloric acid was added to decompose the magnesium oleate in the oil. The flasks were heated to 70 C. on a water bath and maintained at this temperature for twenty-four hours or more, with frequent agitation of the contents to insure complete decomposition of the magnesium soap. At the end of this period, the aqueous layer containing the magnesium as magnesium chloride was separated from the oil layer by filtration through a wet filter paper, and the oil and flask were washed several times with distilled water to remove all magnesium chloride and hydrochloric acid. The

Transfer of Magnesium from Aqueous to Oil Phase, and of Hydrogen from Oil to Aqueous Phase, under Different Conditions

	Initial Conditions			Conditions at Equilibrium				
Series	Gm. of Oleic Acid	p_H	Gm. Mg per Liter (Aq.)	p_H	Gm. Mg per Liter (Aq.)	Gm. Mg Oleate (Oil)	Lowering of p_H	Percentage of Mg Lost from Aqueous Solution
II (1)	1.756	7.53	0.0203	6.31	0.0135	0.0282	1.22	33.7
II (2)	1.751	7.53	0.0203	6.26	0.0131	0.0345	1.27	35.6
III (4)	1.745	7.85	0.0404	6.23	0.0259	0.0739	1.62	36.0
III (5)	1.760	7.85	0.0404	6.22	0.0250	0.0639	1.63	38.2
IV (7)	3.561	6.68	0.0450	6.00	0.0353	0.0457	0.68	21.6
IV (8)	3.510	6.68	0.0450	6.00	0.0343	0.0488	0.68	23.8
V (10)	0.9793	7.47	0.1001	6.13	0.0656	0.166	1.34	34.5
V (11)	1.7953	7.47	0.1001	6.06	0.0642	0.172	1.41	35.8
V (12)	3.7535	7.47	0.1001	6.00	0.0634	0.182	1.47	36.6
VII (17)	0.7950	7.41	0.0526	6.56	0.0397	0.0612	0.85	24.5
VII (18)	1.5286	7.41	0.0526	6.32	0.0363	0.0756	1.09	31.1
VII (19)	3.0963	7.41	0.0526	6.13	0.0350	0.0837	1.28	33.5
VIII (21)	0.8570	6.64	0.0533	6.08	0.0433	0.0509	0.56	18.8
VIII (22)	0.8445	6.36	0.0533	6.02	0.0477	0.0306	0.34	10.6
VIII (23)	0.8479	6.23	0.0533	5.98	0.0495	0.0186	0.25	7.1
VIII (24)	0.8592	6.10	0.0533	5.91	0.0512	0.0113	0.19	3.9
VIII (25)	0.8473	5.77	0.0533	5.68	0.0529	0.0038	0.09	0.7
IX (26)	0.7801	6.71	0.0673	6.10	0.0522	0.0703	0.61	22.4
IX (28)	0.7870	6.26	0.0675	6.02	0.0617	0.0257	0.24	8.6
IX (29)	0.7852	6.09	0.0678	5.91	0.0643	0.0154	0.18	5.1
IX (30)	0.7837	5.77	0.0678	5.71	0.0669	0.0022	0.06	1.3

total aqueous filtrate was analyzed for magnesium essentially as described in foregoing paragraphs. As the total volume exceeded 50 cc., proportionately greater amounts of hydrochloric acid diluted 1:3 and concentrated ammonium hydroxide were used. The filter paper containing the oil was returned to the 125 cc. Erlenmeyer flask, 50 cc. of neutralized 95 per cent alcohol added, and the oil titrated with standard sodium hydroxide to a phenolphthalein end-point. From the volume of alkali required and the known number of grams of oleic acid equivalent to 1 cc. of sodium hydroxide, the weight of oleic acid analyzed was calculated.

RESULTS

The results of several series of experiments are given in the table. In each pair of the first three series, II, III and IV, the variables (the

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initial concentration of magnesium, the p_H and volume of the aqueous phase and the weight of the oleic acid) were as nearly constant as possible. The agreement between the analyses of the pairs indicates the reproducibility of the results: a difference of from 0 to 0.05 in p_H at equilibrium and a difference of from 1.9 to 2.2 in the percentage of magnesium lost from the aqueous phase to form magnesium soap in the oil phase. The percentage of magnesium lost from the aqueous phase varied from about 22 per cent in series IV to about 37 per cent in series III, with an accompanying lowering of the p_H of 0.68 unit in series IV and of from 1.62 to 1.63 units in series III.

In series V and VII, all variables were held constant initially except the amount of oleic acid. In series VII, for example, as the weight of oleic acid was increased from bottle 17 to 19 the percentage of magnesium lost from the aqueous solution increased from 24.5 to 33.5 per cent and the final p_H fell from 6.56 to 6.13.

In series VIII and IX, all variables except the initial p_H of the aqueous phase were held nearly constant. In series VIII, when the initial p_H was lowered successively from 6.64 in bottle 21 to 5.77 in bottle 25, the percentage of magnesium lost from the aqueous solution decreased successively from 18.8 to approximately 0.7 per cent with an accompanying successive lowering of the p_H from 0.56 to only 0.09 unit. The results of series IX were similar.

COMMENT

The results demonstrate conclusively that under the conditions of the experiments a considerable percentage of magnesium is lost from a dilute solution of magnesium acid phosphate ($MgHPO_4$) and is converted into magnesium oleate, which dissolves in the excess of oleic acid present. Simultaneously, hydrogen ions are transferred from the oleic acid to the aqueous solution.

In accord with the principle of LeChatelier,[11] the amount of magnesium removed from the aqueous phase and the accompanying p_H change increase as the amount of oleic acid employed is increased. Likewise, as the initial concentration of hydrogen ions is increased, i. e., as the p_H is lowered, the equilibrium expressed in equation 2 is displaced more and more to the left. Less magnesium leaves the aqueous solution, and a smaller additional quantity of hydrogen ions is formed. When the initial p_H has been lowered to about 5.7, a dilute aqueous solution of $MgHPO_4$ plus $Mg(H_2PO_4)_2$ is so acid that very little reaction with oleic acid occurs.

11. When a "strain," such as an excess of a reactant, is applied to a system, the system reacts in such a way as partly to remove the "strain."

The exchange of ions at the interphase of water and fatty acids demonstrated by these chemical analyses serves to explain how reactions occur in tissues between immiscible hydrolyzing fats and basic substances in aqueous systems. The importance of this reaction in normal physiologic functions has not been investigated, but in pathologic conditions, such as fat necrosis, injury of fat tissues and deposition of fat, an explanation is provided for the gradual accumulation of magnesium, calcium and other basic elements in tissues rich in fat.

SUMMARY

A study was made of the heterogeneous equilibrium

$$\underset{(Aq.)}{Mg^{++}} + 2\underset{(oil)}{C_{17}H_{33}COOH} \rightleftarrows \underset{(oil)}{Mg(C_{17}H_{33}COO)_2} + \underset{(Aq.)}{2H^{+}}$$

using 200 cc. portions of aqueous solutions of magnesium acid phosphate ($MgHPO_4$) containing from 0.02 to 0.1 Gm. of magnesium per liter, with p_H from 5.7 to 7.9. Little reaction with oleic acid occurred at p_H 5.7, but as the p_H was increased a corresponding larger percentage of magnesium was transferred from the aqueous solution to form magnesium oleate, dissolved in the excess of oleic acid present. Hydrogen ions at the same time left the oil phase and appeared in the aqueous phase, lowering the p_H of the aqueous phase. The displacement of the equilibrium followed the law of LeChatelier, being dependent on the p_H and concentration of magnesium in the aqueous phase and on the relative amounts of the two phases.

This reaction between oleic acid and alkaline aqueous solutions of magnesium provides an explanation for the accumulation of magnesium, calcium and other bases in pathologic tissues rich in fats.

EXPERIMENTAL EMBOLIC GLOMERULONEPHRITIS PRODUCED WITH HUMAN FAT, FATTY ACIDS AND CALCIUM SOAPS

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During a study of rabbit tissues that was focused primarily on the pulmonary lesions produced by intravenous injections of fats and soaps, Hirsch [1] observed tissue reactions in the glomerular tufts of the kidneys about droplets of fat which had passed through the pulmonary circulation of these animals and lodged in the glomerular capillaries. The reactive changes caused by these fat emboli in the glomeruli suggested some of the alterations which occur in kidneys with Bright's disease.

Many who have made experimental studies in animals, especially rabbits, have described glomerular reactions caused by bacteria or their products in sensitized [2] and nonsensitized [3] animals, by nephrotoxic substances [4] and by various other agents.[5] There seems to have been no investigation of the effects of embolic lipid material which, in disturbed or in the usual phases of metabolism, circulates in droplet form in the blood and may lodge in the capillary tufts of the glomeruli. The intriguing element of the glomerular reactions is the circumstance that the agent itself during removal or destruction stimulates tissue responses. In the older lesions, where the lipid has been dispersed, only some tissue reaction or scar remains.

MATERIALS AND METHODS

Human fats rendered from peritoneal and retroperitoneal depots of the body were used in these experiments (1) alone, (2) mixed with aliquots of oleic acid or stearic acid or both, the mixture being then neutralized by contact with aqueous

From the Henry Baird Favill Laboratory of St. Luke's Hospital, Chicago, John Jay Borland Fellowship for Clinical Research, and the Department of Pathology of the University of Alabama Medical School, University, Ala.

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solutions of calcium hydroxide, and (3) mixed with acids of hydrolyzed human fat, the mixture being neutralized with calcium hydroxide. In several experiments, oleic acid or liquid petrolatum was used. A 3 cc. amount of the lipid was triturated with 0.75 Gm. of powdered acacia, and about 27 cc. of sterile distilled water was added slowly. The large dilution with water produced an uneven suspension, which was emulsified further by suction into and expulsion from a 10 cc. Luer syringe. The emulsion was transferred to a test tube, where after fifteen minutes the large droplets had collected in a surface layer. The lower layer, containing finely dispersed droplets ranging between 0.06 to 0.003 mm., was sterilized in an autoclave under 15 pounds (6.8 Kg.) of pressure for twenty minutes. One cubic centimeter of emulsion contained about 0.05 cc. of emulsified fat. Dogs were anesthetized with pentobarbital sodium; rabbits, with ether. One renal artery was exposed by a retroperitoneal incision.[6] The emulsion was injected slowly into this renal artery through a 26 gage hypodermic needle: on the average, 5 cc. in dogs and 1 cc. in rabbits. A single injection was made, and the undisturbed kidney on the opposite side served as a control. The animals were killed with ether. Tissues from each kidney were fixed in Zenker's solution and solution of formaldehyde U. S. P. (1:10). Sections of tissue fixed in Zenker's solution and embedded in paraffin were cut 6 to 10 mm. thick and stained with hematoxylin and eosin, azocarmine-aniline blue-orange G (Heidenhain's modification of Mallory's aniline blue stain),[7] aniline blue-orange G (the Lee Brown stain)[7] and lithium silver.[8] Those fixed in solution of formaldehyde were sectioned by the freezing method and were stained with scarlet red.

GENERAL OBSERVATIONS

Fifty dogs and forty rabbits were used in the experiments. They lived from ten to thirty days after being submitted to the injection of emulsified lipid into one renal artery. The following descriptions are of kidneys removed from ten to thirty days after the injection unless otherwise stated.

The gross changes in the kidney depended on the quantity and quality of the material injected into the renal artery. A large amount of emulsion, regardless of its chemical composition, caused anemia, fatty changes and granular contraction. A small amount produced only pallor of the tissues. The granular contraction of the kidney increased as the animal lived longer after the injection. Small kidneys from animals living the maximal periods after the injection were much firmer than those from animals living a short time. Compensatory hypertrophy of the opposite kidney occurred with contraction of the injured kidney. A large volume of a coarse emulsion produced extensive necrosis. Thrombosis of the renal artery happened rarely.

Oleic acid, a necrosing agent, rapidly produced renal damage and small cortical hemorrhages. Neutral fat to which oleic or stearic acids or both had been added and the mixture then neutralized with calcium hydroxide caused similar but less rapid and less marked alterations. Human fat produced changes slowly over a longer time. Liquid petrolatum had the least effect. Human fat and liquid petrolatum seldom caused hemorrhages. The tissue reactions in kidneys of rabbits were more marked than those in dogs. The emulsified fat droplets were carried into the glomerular loops, from one to eight in each tuft.

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6. Miller, E. M., and Apfelbach, C. W.: Arch. Path. **4**:192, 1927.
7. McGregor, L.: Am. J. Path. **5**:545, 1929.
8. Laidlaw, G. P.: Am. J. Path. **5**:239, 1929.

MICROSCOPIC OBSERVATIONS

Unless otherwise stated, the following descriptions of tissues were made from sections stained with azocarmine-aniline blue-orange G and aniline blue-orange G.

Human Fat.—A proliferation of cells around fat droplets in the glomerular tufts occurred within ten days (fig. 1). Most of these cells were of moderate size, with indistinct cell outlines, and had round vesicular nuclei with chromatin granules. A few spindle cells had abundant cytoplasm and elongated nuclei. These accumulations varied from a few cells to dense aggregates, and were within the basement membrane, which occasionally was thickened. Fine and a few coarse collagenous fibrils extended between the cells. The fibrils were more abundant near the basement membrane; when thick, they enclosed cell nuclei. The lumens of a few glomerular capillaries were filled with fibrin mixed with erythrocytes. The basement membrane beyond the proliferated cells was slightly thickened and sinuous. Some focal proliferations of cells in the glomerular tufts were without fat droplets (fig. 1). The fat had been removed by the tissues or the droplets did not lie in the plane sectioned, for the lesions were similar to others associated with fat droplets. There were no polymorphonuclear leukocyte exudates or proliferations of epithelium. The lining cells of tubules associated with the modified glomeruli were atrophic or necrotic when the glomerular obstruction was marked. When the obstruction was slight, the tubule cells were unaltered except for fatty changes. With severe tubular damage, the interstitial collagenous and reticulum fibers were increased and the stroma had lymphocyte infiltrations. The lumens of the tubules associated with damaged glomeruli contained albuminous material. The arteries and veins were unchanged.

Only a few vacuoles remained in the glomerular tufts of the animals thirty days after the injection. Proliferated cells in the tufts were encapsulated or enmeshed by coarse collagenous fibers (fig. 2). All of the capillaries in some tufts were obliterated; only portions in others. Some of these glomerular lesions resembled those of human nephrosclerosis. The collagenous and reticulum fibers surrounding the tubules were thickened.

Human Fat (27 Cc.) and Oleic Acid (3 Cc.) Neutralized over an Aqueous Solution of Calcium Hydroxide.—Scattered glomeruli with fat droplets were present in animals ten days after the injection of this emulsion. Endothelial cells had grown about the vacuoles, and there was a marked increase of collagenous tissue, which compressed the glomerular capillaries (fig. 3). Many glomeruli without vacuoles had these reactive tissue changes; some tufts were scarred completely. The basement membrane of these tufts was indistinct or swollen. The alterations of the tubules and the increase of the interstitial tissues were commensurate with the damage in the tufts.

After one month the fat droplets were surrounded by considerable collagenous material, in which the endothelial cells were embedded. Adhesions occasionally had formed between the glomerular capillaries and Bowman's capsule. Reticulum fibers were present in glomeruli with abundant lipid material.

Human Fat (29 Cc.) and Stearic Acid (1 Cc.) Neutralized over an Aqueous Solution of Calcium Hydroxide.—The changes in the injured kidneys after ten days resembled those produced by human fat and oleic acid, except that the tissue reactions were not so marked. Glomerular tufts, with or without fat droplets, often had a region with proliferation of endothelial cells. Various amounts of coarse collagenous fibers were mingled with these. Most of the individual cells were moderate in size and had vesicular nuclei. A few were fusiform, with abundant cytoplasm. The basement membrane of some was thickened; the pro-

liferated cells, especially those of peripheral tufts, were within the basement membrane. The capillaries in the unchanged portions of the glomeruli were dilated with erythrocytes.

The glomeruli after thirty days were replaced completely or partially by collagenous material enclosing cell nuclei. Many tufts were shrunken, leaving large capsular spaces. Adhesions occasionally were present. Some partially hyalinized

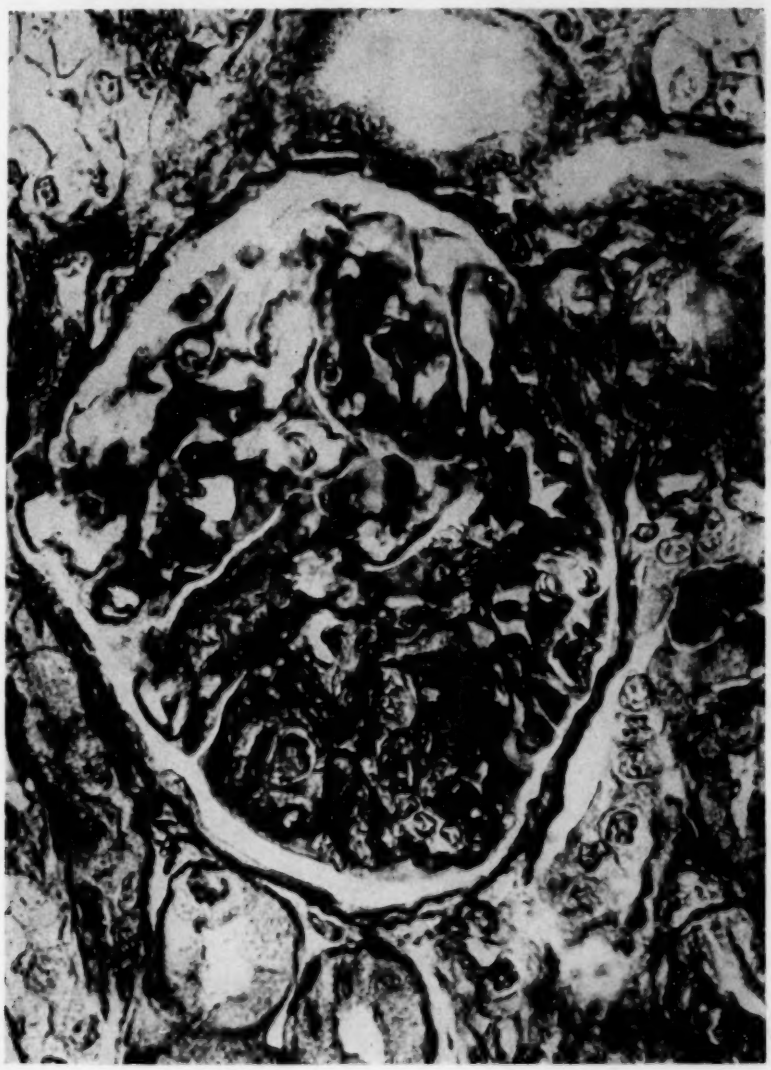

Fig. 1.—Photomicrograph illustrating the hyperplasia of endothelial cells in a glomerular tuft of a rabbit's kidney ten days after an emulsion of human fat had been injected into the renal artery. Near the center of the tuft is a large fusiform cell. (× 920.)

glomeruli had patent capillaries along their peripheries. The reticulum fibers of the interstitial tissues about the corresponding tubules of damaged glomeruli were thickened.

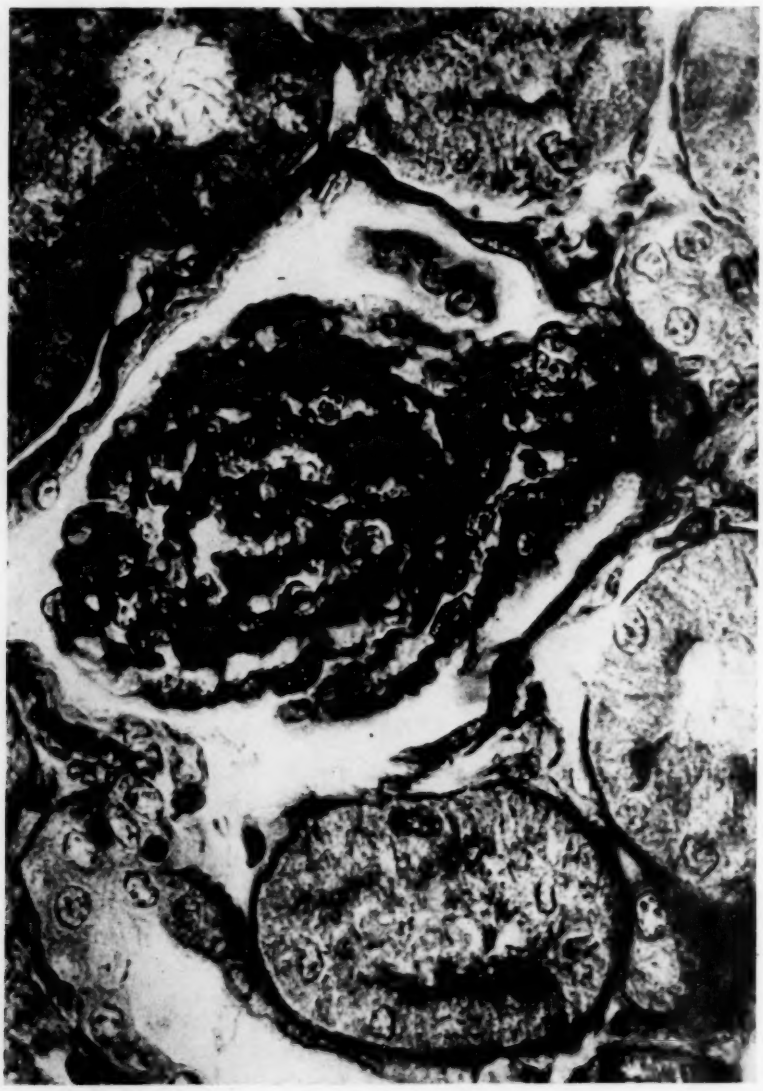

Fig. 2.—Photomicrograph of a glomerulus (dog) one month after an emulsion of human fat had been injected into the renal artery. The tuft is shrunken and markedly distorted by thick collagenous fibers. (× 920.)

Human Fat (25 Cc.) and Oleic Acid (4 Cc.) and Stearic Acid (1 Cc.) Neutralized over an Aqueous Solution of Calcium Hydroxide.—The changes in the kidneys after ten days consisted of endothelial proliferation and collagenous tis-

sue formations between these cells. Small capillaries were present in some collagenous masses. After thirty days the glomeruli were replaced partially or entirely by collagenous material with a few nuclei; many were pyknotic. Other chronic alterations have been described.

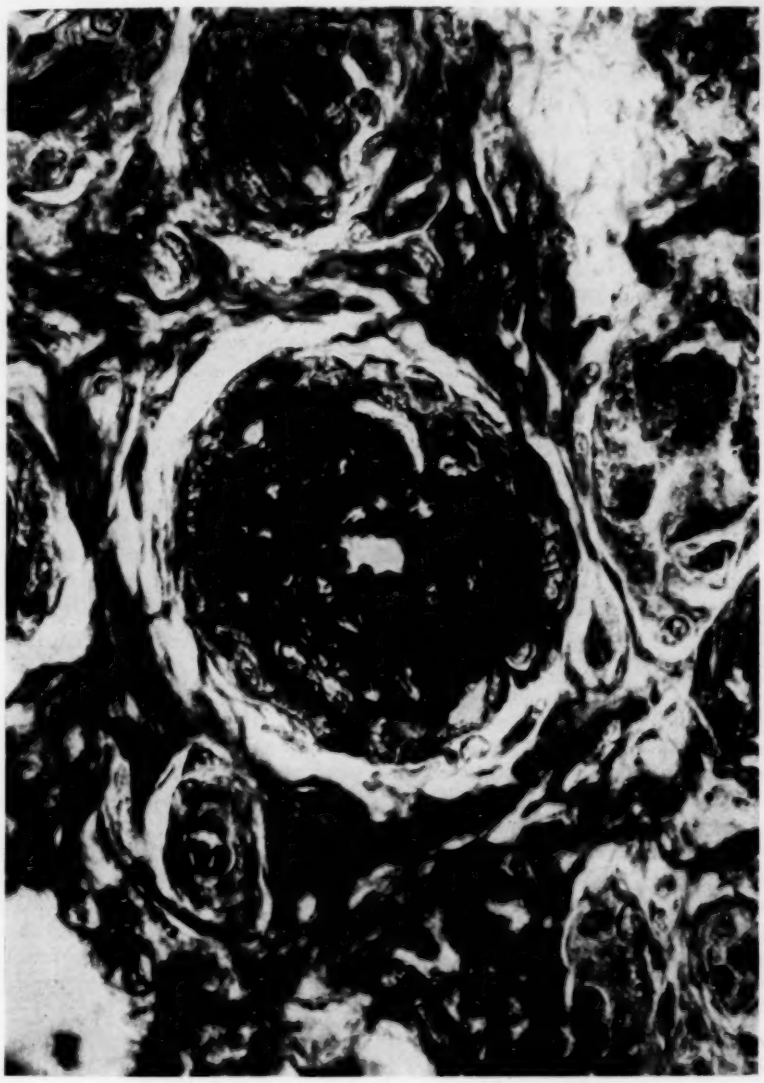

Fig. 3.—Photomicrograph of tissues in the contracted granular kidney of a dog. An emulsion of human fat and calcium oleate had been injected into the renal artery ten days before death. Note the marked increase of collagenous connective tissues in the glomerular tuft and elsewhere. (× 920.)

Human Fat (27 Cc.) and the Fatty Acids of Hydrolyzed Human Fat (3 Cc.) Neutralized over an Aqueous Solution of Calcium Hydroxide.—After ten days the glomerular tufts had a few vacuoles, which were surrounded by proliferated endothelial cells. The number of cells varied, but the endothelial proliferation was greater than with human fat alone. Intercellular collagenous fibers were observed between the proliferated cells and were most numerous near the basement membrane. The endothelial hyperplasia was much more than the increase in collagenous fibers. There was some hyperplasia of the capsular and glomerular epithelium. The tubular and interstitial tissues were damaged in proportion to the amount of glomerular change.

After one month only a few glomerular tufts had fat droplets. The collagenous material was increased; this enclosed endothelial cell nuclei. A few glomeruli were completely scarred, others partially. The interstitial tissues were increased; the tubular changes resembled those described.

Oleic Acid Alone.—This material caused marked changes. After three days the endothelial cells were swollen, and many were necrotic. The glomerular tufts were dilated. Where the endothelial swelling did not cause obstruction, the lumens were filled with erythrocytes.

After ten days, many glomeruli were replaced by collagenous tissue enclosing endothelial cells; others had tufts with an increased number of endothelial cells and varying amounts of collagenous fibers. In these the collagen often was centrally located; a few fibers seemed to be continuous with large fusiform cells. Some glomeruli with slight swelling of the endothelial cells had dilated tufts. The glomerular and capsular epithelium occasionally had proliferated, and the two layers were often adherent. There were many hemorrhages.

The glomeruli were hyalinized after thirty days. The collagenous fibers were coarse and enclosed a few cell nuclei. There were many adhesions between the tufts and the capsules; often the capsular space was obliterated. Most of the hemorrhages had been absorbed. The damage to the tubules and the increase of the interstitial collagenous and reticulum fibers were marked in all kidneys that received the oleic acid.

Liquid Petrolatum.—The glomerular tufts in animals ten days after the injection of this material contained many oil droplets. Endothelial cell proliferation was absent about many of the droplets and where present was slight. The basement membrane and glomerular epithelium were unchanged. The tubules associated with glomeruli that had considerable obstruction were shrunken, and the interstitial tissues about them were increased.

The glomeruli in kidneys of animals killed one month after the injection had many droplets. Proliferated endothelial cells were present about many of the droplets, ranging from one to three layers; a few of the proliferated cells contained emulsified material. The cellular proliferation was focal, and little collagenous material was associated with these cells. Tuft tissues away from the droplet were as a rule unchanged. No hyalinized glomeruli or glomerular adhesions were observed.

Acacia Alone.—Solutions of acacia in the amounts and proportions used in preparing the fat emulsions (2.5 per cent) and injected into the renal arteries of dogs and rabbits produced within ten days a few small foci of endothelial proliferation. Stronger solutions of acacia (from 15 to 20 per cent) were viscous, and when injected into the renal arteries of the animals they produced definite changes. Grossly the kidney was slightly contracted; microscopically the main changes were in the tubules and interstitial tissues, and these consisted of atrophy of the tubular epithelium and in some tubules accumulation of albuminous or cast-

like material in the lumens. The connective tissues about these structures were increased. Some glomerular tufts exhibited slight endothelial proliferation. Many glomeruli were without hyperplasia. The basement membrane was thickened slightly, and a few intercellular fibers were noted.

The amount of acacia used in the experiments was constant and corresponded to the smaller dilution mentioned. A comparison of the lesions produced by the fat emulsified with acacia and those caused by acacia alone leads to the conclusion that the damage to the glomerular tissues was due mainly to the fat and that possibly only minimal alteration was caused by the emulsifying agent. The changes with huge quantities of acacia seemed to be due to obstruction of the glomerular capillaries by the viscous material. The substance had some irritating effect on the endothelial cells.

SUMMARY OF OBSERVATIONS

Emulsions of human fat injected into renal arteries of dogs and rabbits, one artery being used in each animal, affected the involved kidneys in two respects: The lipids stimulated tissue reactions in the glomeruli, the nature of which depended on the chemical composition of the materials injected. The obstruction of the glomerular tufts by the droplets, the reactive tissues or both caused atrophy, necrosis or fatty changes of the cells lining the tubules and growth of interstitial connective tissue.

A strong irritant, oleic acid caused swelling and necrosis of the endothelial cells and marked growth of collagenous tissue,[9] which quickly obliterated or replaced the usual glomerular structures. The fatty acid disappeared rapidly from the tissues and, no doubt, caused the growth of collagenous tissues and necrosis of the endothelium. The response of the glomerular tufts was mainly the growth of a large amount of collagenous substance. The necrosing effect of oleic acid prevented marked endothelial proliferation.

A weak irritant, such as liquid petrolatum, caused a different glomerular response. After many days, there was hyperplasia of the endothelial cells. These cells were associated with only a few collagenous fibers. The cellular response to this material was local and occurred directly about the droplets of liquid petrolatum.

Human fat was a moderate irritant in the tissues of the glomerular tufts. The fat droplets in the glomerular tufts were enclosed by proliferated endothelial cells, associated with some fine and coarse fibers. The droplets disappeared from the tissues after a variable length of time. In the older lesions the collagenous fibers were thicker and enclosed cell nuclei. Human fat containing oleic acid neutralized with calcium hydroxide was a strong irritant but not so rapid in its effect

9. It was not my purpose to determine the source of this tissue. Though the fibers are called collagenous, their source may have been from structures other than the fibroblasts.

as oleic acid. This lipid material caused some proliferation of endothelial cells, and considerable amounts of collagenous material encapsulated these cells. The glomerular structures ultimately were fibrous or hyaline scars. These changes were longer in evolution than those caused by oleic acid.

Human fat containing stearic acid neutralized with calcium hydroxide produced reactions of glomerular tissue similar to but slightly less severe than those caused by the human fat-oleic acid mixture. Human fat containing both oleic and stearic acid neutralized with calcium hydroxide acted more rapidly and with slightly greater damaging effects than the human fat mixtures containing only one soap.

The response of glomerular tissues to these substances was of two types, depending on the nature of the damaging agents. One type of response was the endothelial proliferation, the other was the production of collagenous tissue. Weak irritants caused hyperplasia of the endothelial cells of the glomerular tufts and minimal production of collagen. Strong irritants caused endothelial swelling and more marked formation of collagenous tissue. Mild or moderately strong irritants produced hyperplasia of endothelial cells and moderate growth of collagenous tissue. The strength of the irritant seemed to determine the speed with which the lesions were produced. The tissue response was sometimes focal and caused dilatation of capillaries elsewhere in the tuft.

COMMENT

The fats introduced into the renal arteries of the rabbits and dogs were irritants,[1] and the tissue responses in the glomeruli to the fat droplets, especially in the early stages, resembled those of diffuse glomerulonephritis. Some of the older lesions simulated those in nephrosclerosis. McGregor [10] and Bell [11] stated that the initial change in diffuse glomerulonephritis is a proliferation of the endothelial cells of the glomerular capillaries. McGregor described hyaline fibers that appeared between the proliferated cells. These fibers increased in size and number, fused and gradually enclosed the endothelial cells. Finally, the capillary of the tuft became a few flattened epithelial cells with a basement membrane, and the lumen was completely filled with a hyaline fibrous mass enclosing a few nuclei. Adjacent loops fused and the fibers contracted until most of the cells were eliminated.

MacCallum [12] stated that in some forms of nephritis the essential glomerular change is not proliferation of the capillary endothelium. The capillary lumens may contain masses of fibrin, erythrocytes and

10. McGregor, L.: Am. J. Path. **5**:559, 1929.

11. Bell, E. T.: Am. J. Path. **12**:801, 1936.

12. MacCallum, W. G.: Bull. Johns Hopkins Hosp. **55**:416, 1934; Textbook of Pathology, ed. 6, Philadelphia, W. B. Saunders Company, 1936, p. 272.

leukocytes, which obstruct the circulation through the tuft. The glomerular capillaries in some forms of subacute nephritis and those of longer duration were displaced to the periphery by the growth of new tissues in the central portion of the loop. The capillaries were narrowed, patent and without endothelial changes. According to MacCallum, in nephritis new tissues surround the capillary and separate it from the basement membrane. The greater mass in the center of the loop displaces the capillary outward. In the acute forms, this material is a meshwork with some fluid and leukocytes. Later it becomes hyaline, and the few connective tissue cells normally present in the glomerular tuft proliferate and extend into this formless material between the capillaries. Such alterations are considered evidence of the connective tissue charcter of the material.

Kimmelstiel and Wilson [13] described glomerular lesions in the kidneys of patients with diabetes as glomerulosclerosis. These lesions occurred in the intracapillary form of glomerulonephritis. According to these authors, the essential change was hyalinization of the capillary wall. The central hyalinization of the glomerulus surrounded by a ring of patent capillaries was a change superimposed and independent of capillary hyalinization. In the extracapillary form of glomerulonephritis, these authors confirmed and extended the observations of McGregor: They observed splitting of the basement membrane and thickening of the intercapillary connective tissue.

The results of the experiments cited indicate that the response to the glomerular injury is twofold, depending on the strength of the irritant. The first alteration is an endothelial proliferation associated with some growth of intercellular fibers; the second is a marked production of collagenous tissue about the endothelial cells. The changes noted by McGregor and Bell correspond to the first variety. Those of the second type resemble in certain respects lesions described by MacCallum. Perhaps the differences in the lesions described by these authors might be explained on the basis that strong irritants and milder irritants cause distinct tissue responses. Possibly the irritant producing the intracapillary glomerulonephritis in the lesions described by Kimmelstiel and Wilson was stronger than that causing the intercapillary form.

Apparently the role of fats as a possible etiologic agent in Bright's disease has never been suggested. These lipids and their soaps produce in the kidneys of dogs and rabbits lesions similar to those seen in the diffuse glomerulonephritis of patients. Since emulsified fats circulate in the blood and lipemia occurs in certain diseases, there is the possibility that the fat may have a noxious effect on the glomerular tufts of the kidneys. The emulsified material lodged in the tufts or in contact

13. Kimmelstiel, P., and Wilson, C.: Am. J. Path. **12**:83. 1936.

with the walls produces irritating effects. In the late stages of the lesions only scars or hyperplastic tissues remain; there is no trace of the etiologic agent.

SUMMARY

Lesions similar to those seen in diffuse glomerulonephritis in man were produced in the kidneys of dogs and rabbits by injecting into the renal arteries, emulsified oleic acid, liquid petrolatum and human fat, alone or containing calcium soaps.

Two varieties of glomerular lesions were produced, dependent on the chemical composition of the fat. Mild fat irritants caused proliferation of endothelial cells and minimal growth of collagenous tissue. Strong irritants produced glomerular reactions in which there was swelling of endothelial cells and marked production of collagenous substance. Moderate irritants caused proliferation of endothelial cells and production of collagen.

The various types of lesions in kidneys from patients with glomerulonephritis may represent alterations in response to irritants of different strengths. Fat may be an etiologic agent in the renal changes of Bright's disease.

EXPERIMENTAL TISSUE LESIONS WITH MIXTURES OF HUMAN FAT, SOAPS AND CHOLESTEROL

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Fats in the body are reduced, as a rule, by hydrolysis and oxidation during metabolism to products which are removed without causing an inflammatory reaction of the tissues. Variations from these normal sequences of fat disposal, as with fat necrosis, injuries of fatty tissues and fat deposition, provide opportunity for unusual chemical reactions in the fats and for the entrance into the solvent fat of tissue substances slightly soluble or insoluble in the aqueous fluids of the body. These, unless destroyed as the solvent is gradually removed, remain as irritants to arouse an inflammatory response. References to such reactions in human tissues have been published,[1] as well as a report of embolic lesions produced experimentally in the lungs of rabbits with human fat containing the calcium, strontium and barium soaps of oleic and stearic acids. These soaps were formed in the human fat by the neutralization of added fatty acids in contact with aqueous solutions of the hydroxides of these bases. Chemical analyses have demonstrated a reaction between fatty acids layered on aqueous solutions of bases, with an escape of base ions from the water into the oil phase and of hydrogen ions from the fatty acids into the aqueous medium.[2]

Tissue reactions aroused in rabbits by other human fat substances, not included in the first study but forming an integral part thereof, have been investigated. The substances mixed with human fat were: cholesterol (extracted from human biliary concretions and recrystallized); cholesterol and oleic acid; cholesterol and stearic acid, and cholesterol and oleic or stearic acid, neutralized with aqueous solutions of calcium hydroxide. Also investigated were suspensions in human fat of the calcium, strontium and barium soaps of oleic and stearic acids and suspensions in human fat of the corresponding soaps of hydrolyzed human fat.

EXPERIMENTS

Human Fat[3] *with 4 per Cent Dissolved Cholesterol.*—This is approximately a saturated solution of cholesterol in human fat at 37.5 C. Each of three rabbits

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From the Henry Baird Favill Laboratory of St. Luke's Hospital.

1. Hirsch, E. F.: Arch. Path. **21**:765, 1936.
2. Hirsch.[1] Hartsuch, P. J.: Arch. Path., this issue, p. 17.
3. The human fat and oleic acid were measured volumetrically; the stearic acid and cholesterol, gravimetrically.

received intravenously an injection of from 0.2 to 0.5 cc. each week for two months and then 0.6 cc. weekly for two more months. The total cholesterol content of the blood serum of each rabbit was determined during the last month on the second and third days after the intravenous injection. The six determinations ranged between 28.1 and 50.7 mg. per hundred cubic centimeters of serum. There were no gross deposits of fat in the aorta. About small and large branches of the pulmonary artery and about capillaries were a few infiltrations of lymphocytes, plasma cells and eosinophil leukocytes. Some pneumonic regions had similar exudates and hemorrhages. There were a few small masses of fibroblastic granulation tissues. The lungs of one rabbit had several old fibrous lesions including one or more foreign body giant cells with acicular slits, indicating that some of the cholesterol material had crystallized from the fat solvent and had been encapsulated.

Human Fat (17 Cc.), Oleic Acid (16 Cc.) and Cholesterol (13 per Cent).—At 37.5 C. this mixture was approximately saturated with cholesterol. Each of two rabbits received from 0.3 to 0.4 cc. intravenously once a week for three months. Six determinations of the total cholesterol content of the serum during the last five weeks ranged between 24.8 and 45.5 mg. per hundred cubic centimeters in one rabbit and between 33.7 and 84.7 mg. in the other. The lungs of these two rabbits had a few small foci of lymphocytes, plasma cells, eosinophil leukocytes and occasionally a small giant cell. Scattered small pneumonic regions contained mainly large mononuclear phagocytes. Each of three other rabbits received thirteen intravenous injections of 0.2 cc. of the fat mixture at the rate of one each second or third day for six weeks. The cholesterol content per hundred cubic centimeters of serum on days between the intravenous injections ranged from 50.4 to 104.7 mg in one rabbit, from 41.2 to 95.3 mg. in the second, and from 35.6 to 113.8 mg. in the third. There was no gradual increase during this time. The aortas of both groups of rabbits had no fat deposits. The pulmonary lesions were slight. The lungs of one rabbit had a few focal lesions containing small giant cells, some with acicular slits, indicating that cholesterol had crystallized from the fat solvent.

Human Fat, Stearic Acid (8.4 per Cent) and Cholesterol (7 per Cent).—This mixture was liquid at 37.5 C. Only one of two rabbits survived ten intravenous injections of from 0.3 to 0.4 cc. given over a period of six weeks. Nine determinations of total serum cholesterol ranged between 24.3 and 73.9 mg. per hundred cubic centimeters. The aorta had no deposits of fat. The lungs had scattered foci of a few mononuclear cells and eosinophil leukocytes. There were small regions of pneumonia, and an occasional lesion contained a foreign body giant cell.

Human Fat (17 Cc.), Oleic Acid (6 Cc.) and Cholesterol (12 per Cent) Layered Over an Aqueous Solution of Calcium Hydroxide at 37.5 C.—This mixture became semisolid and for the purposes of injection was emulsified in sterile physiologic solution of sodium chloride and acacia. The amounts injected could not be measured accurately. One rabbit received thirteen injections during six weeks. The serum cholesterol ranged between 31.2 and 50.8 mg. per hundred cubic centimeters. Another rabbit received five injections in three weeks, and the serum cholesterol in five estimations ranged between 51.5 and 76.6 mg. per hundred cubic centimeters. The lesions in the lungs consisted of chronic granulation tissue thrombi composed of fibroblastic tissues, epithelioid cells and large foreign body giant cells. Some of the giant cells had acicular slits, indicating a crystallization of cholesterol from the solution.

Human Fat Containing 16.8 per Cent Solid Stearic Acid and 14 per Cent Cholesterol, Layered Over Aqueous Calcium Hydroxide Solution at 37.5 C.—This mix-

ture, also, became semisolid and for the purposes of injection was emulsified with sterile physiologic solution of sodium chloride and acacia. One rabbit in three weeks received eight injections; eight estimations of the cholesterol in the serum ranged between 38.6 and 89.7 mg. per hundred cubic centimeters. Another rabbit in two weeks received six injections, and the serum cholesterol ranged between 31.7 and 76.1 mg. per hundred cubic centimeters. The lungs had many nodules of chronic granulation tissue containing large foreign body giant cells, some with slits, indicating that cholesterol had crystallized from solution. Large mural thrombi in the branches of the pulmonary artery consisted of similar granulation tissues.

Human Fat Containing 4 per Cent Cholesterol, Layered Over Calcium Hydroxide Solution at 37.5 C.—This mixture remained liquid at 37.5 C. Each of three rabbits received ten injections of 0.5 cc. during five weeks, and ten determinations of serum cholesterol ranged, respectively, from 27.2 to 54.2 mg., from 32.2 to 55 mg. and from 33.8 to 81.7 mg. per hundred cubic centimeters. The lungs in all rabbits had a few small perivascular and pericapillary scars, lymphocytic infiltrations, large mononuclear cells and a few foreign body giant cells. The tissue reactions were much less in amount than in the two preceding groups.

The calcium soaps of stearic and oleic acids and of hydrolyzed human fat were prepared in aqueous solutions, washed with alcohol, dried and suspended in human fat. Each of two rabbits received seven intravenous injections of from 0.25 to 0.4 cc. of a heavy suspension of calcium stearate in human fat within a month; one was killed three and the other four weeks after the last injection. The lungs had small infiltrations of exudate cells about some of the blood vessels. In the lungs of two rabbits that had received injections of a suspension of calcium oleate in the same way, there were a few scattered foci of chronic granulation tissue or exudates. The lungs of two rabbits that had received injections of a suspension of the calcium soaps of hydrolyzed human fat had perivascular cellular infiltrations and small regions of pneumonic exudates.

The suspensions of strontium and barium soaps of oleic, stearic and human fatty acids produced pulmonary lesions essentially the same as those described in a previous report.[1] Human fat incompletely hydrolyzed with aqueous solutions of calcium, strontium and barium hydroxides produced similar lesions in proportion to the extent of hydrolysis.

COMMENT

The results obtained in these and other [4] studies of the tissue reactions about mixtures of human fats and the products of their hydrolysis indicate that at least three factors, alone or combined, play a role in the causation of the lesions. The first is the intensity of the acidity which develops during hydrolysis by the escape of acid ions from the oil phase into the tissue fluids. When sufficiently intense, this acidity caused necrosis of the tissues. The second factor is the nature of the soap compound formed in or about the oil phase during the chemical reaction between the fatty acid and the surrounding aqueous fluids of the tissues. Soaps slightly soluble or insoluble in water accumulate in the oil system or are precipitated in the tissue fluids, where inflammatory reactions occur dependent in character on the base entering into the soap compound. The soaps least soluble in aqueous solutions apparently produce the

4. Hartsuch.[2] Hagerty, C. S.: Arch. Path., this issue, p. 24.

maximal tissue reactions. The third element in the effects of human fat on tissues comprises the substances dissolved in the oil phase, such as cholesterol, which are insoluble in aqueous solutions. These substances in the usual processes of oxidation may be completely utilized, but with varying conditions are not, and remain as insoluble residues, crystalline or otherwise, when the solvent fats, notably those liquid at body temperature, are removed.

The tissue responses in the lungs of rabbits to the calcium, strontium and barium soaps of oleic and stearic acids and of the fatty acids of hydrolyzed human fat are base specific for each, regardless of the method of formation. The soap compounds of each base cause similar tissue reactions whether formed by the exchange of ions at fatty acid-aqueous interphases, by precipitation from aqueous solutions and suspension in oil or by direct hydrolysis of the human fat material with the hydroxide of the bases.

Human fat approximately saturated (roughly 4 per cent at 37.5 C.) with cholesterol prepared from human gallstones, when injected into the circulating blood of rabbits, is destroyed rapidly with little or no tissue reaction in the lungs and other viscera. Human fat slightly supersaturated with cholesterol at body temperature and injected intravenously stimulates in the lungs fibroblastic lesions containing giant cells with acicular slits, the loci of separated cholesterol crystals. The solvent property of human fat for cholesterol is increased markedly by fatty acids; even such mixtures with a high concentration of cholesterol are rapidly utilized in the circulation of rabbits without appreciable tissue reactions. Similar mixtures supersaturated with cholesterol stimulate fibroblastic lesions containing giant cells with acicular slits, indicating a separation of crystalline cholesterol. Fatty acids and human fat mixtures with a high content of cholesterol, not to saturation, becomes semisolid when in contact with aqueous solutions of calcium hydroxide. These mixtures produce in the lungs of rabbits marked fibroblastic lesions including foreign body giant cells, some with acicular slits (cholesterol crystals).

Intravenous injections of mixtures of human fat with fatty acids or calcium soaps and a high content of cholesterol do not alter appreciably the total cholesterol content of the blood serum of rabbits.

In the evaluation of tissue lesions ascribed to deposits of lipid-cholesterol mixtures, the chemical processes involved in the separation of cholesterol crystals from the solvent medium, namely, the factors leading to supersaturation of the solvent, are important. The chemical disturbances leading to supersaturation with cholesterol in such systems may center not alone on the utilization of the cholesterol material, as Leary [5] proposed, but basically on the hydrolysis and oxidation of the solvent medium.

5. Leary, T.: Arch. Path. **21**:419 and 459, 1936.

SUMMARY

At least three factors, alone or combined, play a role in the causation of tissue lesions about mixtures of human fats and the products of their hydrolysis, namely: (1) the intensity of the acidity that develops during hydrolysis through the escape of hydrogen ions from the oil phase into the tissue fluids, (2) the nature of the soap compound found in or about the oil phase during the chemical reaction between the fatty acids and the surrounding aqueous fluids and (3) tissue substances, among them cholesterol, soluble in the oil phase and insoluble in aqueous solutions.

The effects of the soap compounds are base specific (calcium, strontium, barium) whether formed by the exchange of ions at fatty acid-aqueous interphases, by precipitation from aqueous solutions and suspension in oil or by direct hydrolysis of the human fat material with the hydroxides of the bases.

An approximately saturated solution of cholesterol from human biliary concretions in human fat (roughly 4 per cent at 37.5 C.) is destroyed rapidly in the circulation of rabbits without appreciable lesions in the lungs and other viscera. Human fat slightly supersaturated with cholesterol at body temperature and injected intravenously stimulates in the lungs fibroblastic lesions with foreign body giant cells.

Fatty acids markedly increase the solvent property of human fat for cholesterol. Such mixtures with a high concentration of cholesterol are also rapidly utilized in the circulation of rabbits, without appreciable tissue reactions. Fatty acid-human fat mixtures with a high content of cholesterol become semisolid by contact with aqueous solutions of calcium hydroxide. These semisolid substances produce in rabbits fibroblastic lesions containing foreign body giant cells with acicular slits (the loci of cholesterol crystals).

The total cholesterol content of the blood serum of rabbits is not appreciably increased by intravenous injections of human fat alone or with fatty acids and containing high concentrations of cholesterol.

In evaluating tissue lesions ascribed to deposits of lipid-cholesterol mixtures, the factors leading to supersaturation of the solvent with cholesterol are important. The chemical disturbances leading to supersaturation with cholesterol in such systems may center not on the utilization of the cholesterol material but on the hydrolysis and oxidation of the solvent fat medium.

maximal tissue reactions. The third element in the effects of human fat on tissues comprises the substances dissolved in the oil phase, such as cholesterol, which are insoluble in aqueous solutions. These substances in the usual processes of oxidation may be completely utilized, but with varying conditions are not, and remain as insoluble residues, crystalline or otherwise, when the solvent fats, notably those liquid at body temperature, are removed.

The tissue responses in the lungs of rabbits to the calcium, strontium and barium soaps of oleic and stearic acids and of the fatty acids of hydrolyzed human fat are base specific for each, regardless of the method of formation. The soap compounds of each base cause similar tissue reactions whether formed by the exchange of ions at fatty acid-aqueous interphases, by precipitation from aqueous solutions and suspension in oil or by direct hydrolysis of the human fat material with the hydroxide of the bases.

Human fat approximately saturated (roughly 4 per cent at 37.5 C.) with cholesterol prepared from human gallstones, when injected into the circulating blood of rabbits, is destroyed rapidly with little or no tissue reaction in the lungs and other viscera. Human fat slightly supersaturated with cholesterol at body temperature and injected intravenously stimulates in the lungs fibroblastic lesions containing giant cells with acicular slits, the loci of separated cholesterol crystals. The solvent property of human fat for cholesterol is increased markedly by fatty acids; even such mixtures with a high concentration of cholesterol are rapidly utilized in the circulation of rabbits without appreciable tissue reactions. Similar mixtures supersaturated with cholesterol stimulate fibroblastic lesions containing giant cells with acicular slits, indicating a separation of crystalline cholesterol. Fatty acids and human fat mixtures with a high content of cholesterol, not to saturation, becomes semisolid when in contact with aqueous solutions of calcium hydroxide. These mixtures produce in the lungs of rabbits marked fibroblastic lesions including foreign body giant cells, some with acicular slits (cholesterol crystals).

Intravenous injections of mixtures of human fat with fatty acids or calcium soaps and a high content of cholesterol do not alter appreciably the total cholesterol content of the blood serum of rabbits.

In the evaluation of tissue lesions ascribed to deposits of lipid-cholesterol mixtures, the chemical processes involved in the separation of cholesterol crystals from the solvent medium, namely, the factors leading to supersaturation of the solvent, are important. The chemical disturbances leading to supersaturation with cholesterol in such systems may center not alone on the utilization of the cholesterol material, as Leary [5] proposed, but basically on the hydrolysis and oxidation of the solvent medium.

5. Leary, T.: Arch. Path. **21**:419 and 459, 1936.

SUMMARY

At least three factors, alone or combined, play a role in the causation of tissue lesions about mixtures of human fats and the products of their hydrolysis, namely: (1) the intensity of the acidity that develops during hydrolysis through the escape of hydrogen ions from the oil phase into the tissue fluids, (2) the nature of the soap compound found in or about the oil phase during the chemical reaction between the fatty acids and the surrounding aqueous fluids and (3) tissue substances, among them cholesterol, soluble in the oil phase and insoluble in aqueous solutions.

The effects of the soap compounds are base specific (calcium, strontium, barium) whether formed by the exchange of ions at fatty acid-aqueous interphases, by precipitation from aqueous solutions and suspension in oil or by direct hydrolysis of the human fat material with the hydroxides of the bases.

An approximately saturated solution of cholesterol from human biliary concretions in human fat (roughly 4 per cent at 37.5 C.) is destroyed rapidly in the circulation of rabbits without appreciable lesions in the lungs and other viscera. Human fat slightly supersaturated with cholesterol at body temperature and injected intravenously stimulates in the lungs fibroblastic lesions with foreign body giant cells.

Fatty acids markedly increase the solvent property of human fat for cholesterol. Such mixtures with a high concentration of cholesterol are also rapidly utilized in the circulation of rabbits, without appreciable tissue reactions. Fatty acid-human fat mixtures with a high content of cholesterol become semisolid by contact with aqueous solutions of calcium hydroxide. These semisolid substances produce in rabbits fibroblastic lesions containing foreign body giant cells with acicular slits (the loci of cholesterol crystals).

The total cholesterol content of the blood serum of rabbits is not appreciably increased by intravenous injections of human fat alone or with fatty acids and containing high concentrations of cholesterol.

In evaluating tissue lesions ascribed to deposits of lipid-cholesterol mixtures, the factors leading to supersaturation of the solvent with cholesterol are important. The chemical disturbances leading to supersaturation with cholesterol in such systems may center not on the utilization of the cholesterol material but on the hydrolysis and oxidation of the solvent fat medium.

A CHEMOTROPIC SUBSTANCE DERIVED FROM NORMAL TISSUES

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Since the ameboid movements of polymorphonuclear leukocytes were first observed by Wharton Jones in 1846 the attracting power of various substances have been tested and many theories have been evolved to explain leukocytic behavior. The earlier literature has been reviewed by Wells.[1] Later investigation has centered along three lines: the chemotropism (1) of bacteria or their products, (2) of chemical or physical forces and (3) of tissue substances. Bacterial chemotaxis has been observed frequently and has been established by McCutcheon and his co-workers.[2a] They have found that many bacteria and certain fractions of bacterial substance are strongly chemotactic. Electrophoresis has been held responsible for the emigration of leukocytes toward injured tissues by Abramson.[3] He showed that a proper difference of potential exists between the negatively charged leukocyte and the positively charged tissue surface. Maltaner and Hoppe [4] and Moppett [5] produced some evidence supporting the view that ion concentration is the motivating factor in chemotaxis. Variation in surface tension as the underlying mechanism has received the support of Commandon,[6] Habler and Weber [7] and others. Finally it should be mentioned that certain chemical substances—such as carbohydrates [8]—seem to have a property by which leukocytes are attracted.

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1. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925, p. 275.

2. (*a*) McCutcheon, M., and Dixon, H. M.: Arch. Path. **21**:749, 1936. (*b*) McCutcheon, M.; Dixon, H. M., and Czarnetzky, E. J.: Am. J. M. Sc. **193**:871, 1937; (*c*) Am. J. Physiol. **66**:180, 1923. (*d*) McCutcheon, M.; Wartman, W. B., and Dixon, H. M.: Arch. Path. **17**:607, 1934. (*e*) McCutcheon, M., and Dixon, H. M.: ibid. **19**:679, 1935.

3. Abramson, H. A.: J. Exper. Med. **46**:487, 1927; J. Gen. Physiol. **11**:463, 1928.

4. Maltaner, F., and Hoppe, E.: J. Hyg. **19**:309, 1921.

5. Moppett, W.: Proc. Roy. Soc., London, s.B **106**:175, 1930.

6. Commandon, J.: Compt. rend. Soc. de biol. **82**:1117 and 1305, 1919.

7. Habler, C., and Weber, C.: Klin. Wchnschr. **9**:760, 1930.

8. Grant, C. B., and Chambers, R.: J. Cell. & Comp. Physiol. **8**:1, 1936.

Tissue substances have not received serious consideration as chemotactic agents, although earlier investigators had observed this phenomenon. Buchner [9] found that alkaline albuminates from organs were chemotactic. Massart and Bordet [10] showed chemotropic properties in peritoneal and pleural exudates. Bloch [11] reported positive results with glycerol extracts of organs. Dold [12] demonstrated chemotropism with products of tissue disintegration. More recently Grant and Chambers [13] described chemotaxis toward injured muscle tissue, its water-soluble thermolabile extract and sterile peritoneal exudate. Menkin [14] isolated a crystalline water-soluble substance from sterile pleural exudate which is chemotropic and which he believed to be a product of protein catabolism.

It is well known that chemotropism is manifested in tissues following various types of injuries. These include sterile mechanical or thermal [15] injuries as well as infections. Moon [16] emphasized the importance of substances derived from injured cells as factors initiating the cellular as well as the vascular phenomena of acute inflammation. In this connection it is important to determine whether normal dermal and epidermal tissues contain demonstrable chemotropic substances and to investigate some of the factors which may influence the behavior of leukocytes.

EXPERIMENTAL PROCEDURES

The technic developed by McCutcheon and his co-workers was rigorously followed, except that the handling of tissues was slightly different and that an electric thermostatic warm stage was substituted for the thermostatic box used by them. Normal human skin was obtained from specimens removed surgically and was preserved on ice. The skin was cleansed (if necessary) and cut on a freezing microtome into sections from 5 to 10 microns in thickness. The sections were made parallel to the skin surface, and contact with water was avoided. Microscopic examinations showed that these sections contained all skin layers but consisted mostly of papillary and epidermal tissue. The sections were then pulped by smashing with a clean steel hammer on a polished steel block. A bit of skin pulp was transferred to a coverslip, a drop of fresh human blood was overlaid, the coverslip was carefully inverted on a glass slide and the preparation sealed with petrolatum. Both coverslip and slide were cleansed according to McCutcheon's

9. Buchner, H.: Berl. klin. Wchnschr. **27**:1084, 1890.

10. Massart, J., and Bordet, C.: Ann. Inst. Pasteur **5**:417, 1891.

11. Bloch, G.: Centralbl. f. allg. Path. u. path. Anat. **7**:785, 1896.

12. Dold, H.: Arb. a. d. path. Inst. zu Tübingen **9**:30, 1914.

13. Grant, C. B., and Chambers, R.: J. Cell. & Comp. Physiol. **9**:165, 1936.

14. Menkin, V.: Proc. Soc. Exper. Biol. & Med. **36**:164, 1937.

15. Loos, H. O.: Arch. f. Dermat. u. Syph. **164**:199, 1931; Ztschr. f. d. ges. exper. Med. **75**:463, 1931. Schattenberg, H. J., and Harris, W. H.: Proc. Soc. Exper. Biol. & Med. **29**:269 and 1052, 1931. Moon.[16]

16. Moon, V. H.: Arch. Path. **20**:561, 1935.

method.[2c] The preparation was then placed on an electrically warmed stage and allowed to come to equilibrium before observations were begun. The margin of the particle of skin was brought into the field and the courses of neighboring leukocytes were traced for at least four minutes at intervals of from 30 to 60 seconds through a camera lucida.[2d] Control observations were made in fields remote from the epithelium and in similar preparations made without any chemotropic substance.

Results are expressed according to McCutcheon's "coefficient of chemotaxis" [2e] here interpreted as the difference between the initial and the final *normal,* or *direct,* straight line distance to the attracting surface (i. e., the net approach of the leukocyte) divided by the total path of the leukocyte (figure). The coefficient equals $\frac{AN_1 - BN_2}{AB}$, which varies between the arbitrary limits of $+1$ and -1. The course of 103 leukocytes in a series of twelve preparations, traced and computed as described, gave a mean coefficient of $+0.83$, indicating that dermal tissues contain an actively chemotropic substance.

It was observed early in these experiments that skin pulp from sections which had been soaked in water did not attract leukocytes. Accordingly, in a second

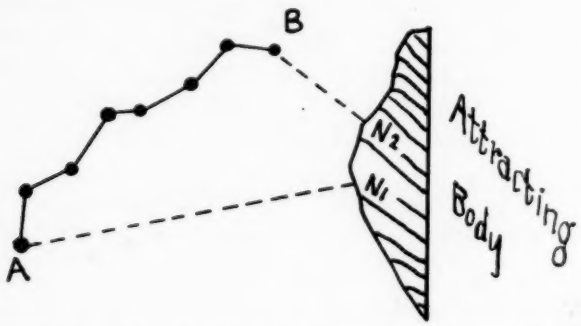

Diagram to show: *AB,* path of leukocyte; AN_1, initial direct distance to attracting body; BN_2, final direct distance.

series of experiments, pulped skin was extracted by heating for fifteen minutes in a large volume of distilled water, repeating this procedure several times with fresh water. This extracted skin was then tested for chemotaxis in the same manner as in the first experiment. The courses of 32 leukocytes in 4 such preparations showed no chemotropic response. Their behavior is indicated by a coefficient of $+0.05$. The first watery extract was then concentrated by evaporation and a small amount of the *extracted* skin pulp was placed in the concentrate for six hours, after which it was tested for chemotaxis. The average course of 17 leukocytes in these preparations gave a coefficient of $+0.77$, showing that the chemotropic substance in dermal tissues is thermostabile and water soluble and that its effects can be demonstrated in watery extracts of such tissues. Further corroborative evidence was obtained by using kaolin as an adsorbing medium, following the suggestion of McCutcheon. Kaolin alone has a coefficient of -0.24 (29 leukocytes), but after it has been soaked in the extract its coefficient becomes $+0.81$ (20 leukocytes). The extract was analyzed qualitatively for certain substances. Negative reactions were found for carbohydrate (Molisch and aniline acetate

tests) and for histamine,[17] but positive reactions were obtained for protein (biuret and xanthoproteic tests) and the free amino-acid group (ninhydrin test).

Since the similarity between histamine and Sir Thomas Lewis' "H-substance" was shown, there have been several attempts to determine whether histamine is chemotactic.[18] Accordingly, observations were made on solutions of histamine phosphate (1:500 and 1:5,000) with use of extracted skin pulp and kaolin as adsorbing mediums. To be certain that the histamine had been adsorbed, the pharmacologic test for the effect of histamine on guinea pig uterus was made on the kaolin-histamine fraction and was found positive. The coefficients obtained were +0.25 (29 leukocytes) for extracted skin as the adsorbent and —0.13 (24 leukocytes) for kaolin. These results indicated little if any chemotropic response.

The effects of salt (ion) concentration and of hydrogen ion concentration (electrophoresis) were studied. The following preparations of purified Difco agar, which was leached with water, alcohol and ether, were tested: a 2 per cent agar gel using distilled water, physiologic solution of sodium chloride and 2 per cent salt solution; a 2 per cent gel adjusted to a p_H of 5.5, 7.4 and 8.5. These preparations of agar showed nearly identical coefficients, all being within a few hundredths of +0.70 (table 2). Kaolin soaked in the following solutions—distilled water, 3 per cent salt solution, tenth-normal sodium hydroxide and tenth-normal hydrochloric acid—was tested for chemotaxis. Again, all preparations gave nearly identical coefficients, the kaolin remaining consistently negatively chemotactic around —0.25 (table 2).

To obtain a comparison with McCutcheon's results a few preparations of Staphylococcus aureus were observed; the courses of 15 leukocytes in two such tests gave a coefficient of +0.65.

COMMENT

It should be stated that this method of determining chemotropism should not be regarded as mathematically exact. First, owing to the fact that the leukocyte can change its shape remarkably, it is difficult to estimate the center of a leukocyte at any particular moment more closely than 1.5 mm. on the camera lucida (equivalent to 2 microns in the apparatus used in the present study). Thus, as the average single measurement is between 10 and 15 microns, the error from this source is approximately from 10 to 15 per cent. Second, the direct line distance, which represents the shortest distance to the attracting substance, is difficult to obtain with certainty when the attracting surface is irregular and when only a small portion of the surface can be seen in the microscopic field at one time. The probable error as calculated according to the method described by Dunn[19] indicates that the data for the coefficients are uniform except when random distribution is expected.

17. Qualitative histamine tests described by F. Axmacher (Biochem. Ztschr. **284**:339, 1936).

18. Wolf, E. P.: J. Exper. Med. **35**:375, 1921; **37**:511, 1923. Bloom, W.: Bull. Johns Hopkins Hosp. **33**:185, 1922. Paul, J. R.: ibid. **32**:20, 1921. Grant, R. T., and Wood, J. E., Jr.: J. Path. & Bact. **31**:1, 1928. Morgan, J. R. E.: Arch. Path. **18**:516, 1934. Moon.[16]

19. Dunn, H. L.: Physiol. Rev. **9**:275, 1929.

The method, while lacking in mathematical precision, affords a convenient means for expressing and tabulating the behavior of leukocytes under various conditions.

By examining table 1, it will be seen that normal human skin when injured and tested in the manner described has a high degree of chemotaxis—a coefficient of + 0.83. A higher degree of chemotaxis is suggested by the median, which is + 0.89. This coefficient compares favorably with that of Staphylococcus aureus (+ 0.65); McCutcheon

TABLE 1.—*Chemotaxis of Injured Human Skin*

Substance	Leukocytes Observed	Experiments	Coefficient	Probable Error
Skin pulp	103	12	+0.83	±0.09
Extracted pulp	32	4	+0.05	±0.32
No. 2 in extract	17	2	+0.77	±0.08
Kaolin	29	3	—0.24	±0.17
Kaolin in extract	20	2	+0.81	±0.10
Staph. aureus	15	2	+0.65	±0.11
Controls	55	6	+0.07	±0.24

TABLE 2.—*Chemotaxis of Variously Prepared Agar and Kaolin*

Substance	Leukocytes Observed	Experiments	Coefficient	Probable Error
2% agar with distilled water	18	2	+0.72	±0.12
2% agar with physiologic solution of sodium chloride	16	2	+0.66	±0.11
2% agar with 2% salt solution	17	2	+0.68	±0.12
2% agar with p_H 5.5	20	2	+0.65	±0.12
2% agar with p_H 7.4	15	2	+0.74	±0.16
2% agar with p_H 8.5	18	2	+0.71	±0.09
Kaolin soaked in distilled water	29	3	—0.24	±0.17
Kaolin soaked in 3% salt solution	17	2	—.033	±0.28
Kaolin soaked in tenth-normal sodium hydroxide	15	2	—0.21	±0.16
Kaolin soaked in tenth-normal hydrochloric acid	18	2	—0.24	±0.21

has reported a higher coefficient (+ 0.80). Thus it appears that the cellular reaction in acute inflammation may depend in part on bacterial substances and in part on substances derived from tissues. These substances can be dissolved out of the skin, for skin extracted with water loses its chemotropic property. This chemotropic substance is not destroyed by heat and can be demonstrated in the watery extract concentrated by evaporation. This result agrees with the finding of Menkin that the chemotropic substance is water soluble and thermostabile. Chemically, the extract is in close agreement with Menkin's crystalline substance, except that it contains protein, whereas his material is protein free. Experiments, not described here, with skin obtained post mortem (more than twelve hours after death) showed distinct diminution of

chemotropism, and with the older specimens, even negative chemotaxis. This suggests that the chemotropic substance disappears, perhaps by autolytic processes, after death.

Histamine in the experiments described showed slight suggestive chemotropic influence. However, the data were widely dispersed about the mean coefficient (probable error of ± 0.30), and hence no conclusions can be drawn.

Experiments made with inorganic materials yielded uniformly negative results. The positive chemotaxis of plain agar and the negative chemotaxis of kaolin were not influenced one way or the other by varying either the hydrogen ion concentration or the salt concentration (table 2). These results call into question the importance of the role that simple physicochemical forces (electrophoresis and concentration gradients) play in the phenomenon of chemotropism. This view is shared by Grant and Chambers,[8] who also described the positive chemotaxis of agar (principally a carbohydrate).

SUMMARY

The tissues of normal human skin contain a substance which is actively chemotropic. This substance (or substances) probably is a factor in determining the behavior of leukocytes following injury to skin. In noninfected injured skin chemotaxis must depend on substances derived from the tissues. Chemotropism in infected areas may depend in part on bacterial substances and in part on substances derived from the injured tissues.

Simple physicochemical forces did not appear to be important factors in chemotropism in these experiments.

CULTURAL STUDIES ON THE RELATIONSHIP OF LYMPHOCYTES TO MONOCYTES AND FIBROBLASTS

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In spite of a vast amount of morphologic and experimental studies, the relationship of lymphocytes to monocytes and fibroblasts has not been definitely established. The numerous opinions on the subject are collected in textbooks of histology and hematology, and the most recent literature on the subject is reviewed in the articles of Maximow,[1] Bloom [2] and Ehrich.[3]

Some workers have maintained that the lymphocytes represent an independent cell type (Aschoff and Kiyono;[4] Herzog;[5] Marchand;[6] Lewis;[7] Clark and Clark [8]) whereas others have maintained that lymphocytes may give rise to monocytes and the latter to fibroblasts. The main proponents of the latter opinion have been Maximow [1] and Bloom;[9] they have described the transformation of small lymphocytes into large lymphocytes, which give rise to monocytes. Pierce [10] described this transformation in cultures of leukemic lymphocytes.

Maximow,[11] Marchand [12] and Downey and Weidenreich [13] believed, with the majority of current workers, that lymphocytes are derived from undifferentiated

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1. Maximow, A. A.: Arch. f. exper. Zellforsch. **5**:169, 1928.
2. Bloom, W., in Hirschfeld, H., and Hittmair, A.: Handbuch der allgemeinen Hämatologie, Berlin, Urban & Schwarzenberg, 1933, vol. 1, p. 1179.
3. Ehrich, W., cited by Lubarsch, O., and von Ostertag, R.: Ergebn. d. allg. Path. u. path. Anat. **29**:1, 1934.
4. Aschoff, L., and Kiyono, K.: Folia haemat. **15**:383, 1913.
5. Herzog, G.: Klin. Wchnschr. **2**:684, 1923.
6. Marchand, F., in Krehl, L., and Marchand, F.: Handbuch der allgemeinen Pathologie, Leipzig, S. Hirzel, 1924, vol. 4, p. 78.
7. Lewis, W. H., in Harvey Lectures, 1925-1926, Baltimore, Williams & Wilkins Company, 1927, p. 77.
8. Clark, E. R., and Clark, E. L.: Am. J. Anat. **46**:149, 1930.
9. Bloom, W.: Arch. f. exper. Zellforsch. **5**:269, 1928.
10. Pierce, M.: Arch. Path. **14**:295, 1932.
11. Maximow, A. A.: Arch. f. mikr. Anat. **97**:283, 1923.
12. Marchand, F.: Verhandl. d. deutsch. path. Gesellsch. **16**:5, 1913.
13. Downey, H., and Weidenreich, F.: Arch. f. mikr. Anat. **80**:306, 1912.

mesenchymal cells, which form a network supporting the blood-forming tissues and are commonly named reticulum cells; but Goldmann,[14] Kiyono[15] and others expressed the belief that reticular cells do not form lymphocytes.

Recently Bloom, surveying the literature,[16] concluded that the evidence is strongly in favor of the identity of the myeloblast and lymphoblast, and of the existence of one common indifferent stem cell, the hemocytoblast, for all elements of the blood. This, he believed, is a large cell with a clear vesicular nucleus and with a narrow rim of basophilic cytoplasm. In lymphoid tissue it differentiates into lymphoid cells and in myeloid tissue into myeloid cells. He called the small lymphocytes inactive hemocytoblasts because under certain conditions this cell differentiates into either large lymphocytes or monocytes.

There are many conflicting opinions regarding the origin of the monocytes. Forkner[17] quoted nineteen different opinions. These do not include the view that monocytes represent an independent cell type, capable of perpetuation by mitotic division. Under different conditions monocytes assume different morphologic characteristics and may resemble other cell types (e. g., large lymphocytes, fibroblasts or endothelial cells), but there is no conclusive evidence that they are related to these cells. The principal theories regarding the origin of monocytes and their main proponents, according to Forkner,[17] are as follows: 1. Monocytes originate from myeloblasts (Ehrlich; Naegeli; Ziegler; Sternberg). 2. They originate from lymphocytes (Maximow; Weidenreich; Bergel; Lang; Bloom). 3. They originate from vascular endothelium (Mallory; McJunkin). 4. They originate from endothelial or reticular cells of blood-forming tissue (Aschoff and Kiyono). 5. They arise from primitive undifferentiated cells (Sabin, Doan and Cunningham; Forkner; Ferrata). It is now generally accepted that monocytes may transform into histiocytes (macrophages) and epithelioid cells (Carrel and Ebeling;[18] M. R. Lewis;[19] Hetherington and Pierce;[20] Dunning and Furth[21]).

The purpose of this study is to investigate the conditions under which lymphocytes are thought to undergo transformation into monocytes and fibroblasts. The most significant of the experimental studies supporting this opinion were made with the aid of tissue cultures, and in some of these experiments material presumably containing lymphocytes alone, namely, lymph of the thoracic duct, was used.

Bloom[9] studied the lymph of twenty-four rabbits, some of which were normal, while others had peritonitis produced experimentally by various irritants. He found that the lymph of the thoracic duct contained very few cells other than lymphocytes of different sizes, but

14. Goldmann, E.: Beitr. z. klin. Chir. **78**:1, 1912

15. Kiyono, K.: Die vitale Karminspeicherung, Jena, Gustav Fischer, 1914.

16. Bloom, W., in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, Inc., 1932, vol. 2, p. 603.

17. Forkner, C. E.: Arch. Int. Med. **53**:1, 1934.

18. Carrel, A., and Ebeling, A. H.: J. Exper. Med. **36**:365, 1922.

19. Lewis, M. R.: Am. J. Path. **1**:91, 1925

20. Hetherington, D. C., and Pierce, E. J.: Arch. f. exper. Zellforsch. **12**:1, 1931.

21. Dunning, H. S., and Furth, J.: Am. J. Path. **11**:895, 1935.

quoted other investigators who found in this lymph from less than 1 to 18 per cent monocytes, the number depending on the methods of staining and the species of animal used.

Lymph was collected by Bloom in a pipet as it poured from a small incision made in the thoracic duct. His technic varied greatly; lymph was obtained from animals with peritonitis produced by different irritants, and five different extracts were used to promote the growth of the cells in vitro. The results are not described in sufficient detail to define the influence of the various factors on the cellular composition of the lymph and on the fate of the cells in vitro.

Bloom found that the lymphocytes formed pseudopods during the first three hours of incubation, and that when exposed to neutral red they contained from 2 to 4 neutral red stained vacuoles. After three hours of incubation, the cells contained a moderate number of neutral red stained vacuoles. Many cells were dead, as indicated by the opacity and rounded shape of the cells and the sharper definition of the nucleus. Most of the cells within the clot were alive. After from five to six hours, an increasing number of cells contained a greater number of neutral red droplets (from 12 to 15), which were confined to one area of the cell and were never found scattered throughout the cell. After six hours the cells were larger. The nucleus was larger and had become indented. In many of the cells the cytoplasm was increased. All cells had not changed; typical lymphocytes were still numerous. When neutral red was added after eight hours of incubation, it was collected, in many of the cells, in the form of rosettes. After from ten to twelve hours, the neutral red droplets were larger but not much more numerous, and the cells were distinctly elongated. Many cells contained numerous highly refractile greenish yellow lipoid droplets situated at the periphery of the cytoplasm in a zone surrounding the neutral red droplets, and they often obscured the nucleus. In cultures made with plasma and embryonic extract a moderate number of lymphocytes became rounded and died. After from twenty to thirty hours, there were large cells with many long processes. After two or three days, the cells were still larger and the cytoplasmic processes longer. The nucleus was relatively small. After three or four days of incubation, the cytoplasm of the large cells lost its lipoid droplets and became finely granular; the cells were then indistinguishable from fibroblasts.

Bloom's work has been repeated with the same results by Bergel;[22] the latter's studies will be discussed after our own results have been presented.

EXPERIMENTAL TECHNIC

In the two series of cultures lymph of the thoracic duct was obtained from normal dogs, and in seventeen series, from rabbits. Ten of these rabbits were normal, six had advanced tuberculosis, and one had been given injections of a growth of Bacterium monocytogenes three weeks before operation.

The animals used were starved for twenty-four hours in order to obtain lymph as free from fat as possible. Blood was obtained from the marginal ear vein, and differential counts were made from dry fixed smears stained with Wright and Giemsa solutions and from supravitally stained preparations. The

22. Bergel, S.: Arch. f. exper. Zellforsch. **9**:269, 1930.

animals were then anesthetized with either sodium barbital U. S. P. (200 mg. per kilogram of body weight) or ether. The thoracic duct was exposed by the operation advocated by Kindwall.[23] For the earlier series of cultures the lymph was obtained in the manner described by Kindwall[23] and Bloom,[9] but later it was found possible to obtain lymph by inserting into the thoracic duct a 27 gage hypodermic needle and drawing the lymph up into a syringe containing heparin (9 parts lymph to 1 part heparin). In some experiments the lymph was allowed to clot immediately. Differential counts were made from dry fixed smears and supravitally stained preparations of the lymph.

The heparinized lymph was centrifugated for ten minutes at 2,000 revolutions per minute, and the upper four fifths of the lymph plasma was discarded. The lowermost part was clotted by the addition of chick embryonic extract. The clotted lymph was then cut up into minute fragments in the presence of Tyrode solution, the fragments were placed on a cover slip, 1 drop of homologous plasma was added, and the mixture clotted by the addition of 1 drop of embryonic extract (1 part of embryonic extract to 9 parts of Tyrode solution). The best cultures were obtained by embedding the lymph clot in plasma diluted with approximately nine times its volume of homologous serum.

Some lymph cultures were prepared by clotting with embryonic extract drops of heparinized lymph placed on cover slips. This procedure insured an even distribution of cells. The changes that occurred in most of the cells after varying periods of incubation could be observed more readily in these cultures than in those prepared from fragments of clot, but the cells lived longer in the latter.

Some lymph was allowed to clot immediately after removal from the thoracic duct, and cultures were prepared in the same manner as with the heparinized centrifugated lymph.

In order to identify the cells and to determine their phagocytic properties, cultures were set up on cover slips that had been evenly coated with fine carbon particles by passage through the smoke of burning wood. To other cultures trypan blue in a 1:2,000 concentration was added. The buffy coat of blood from several normal and several tuberculous rabbits whose lymph was incubated was cultured by the method used for the lymph.

After the cultures of lymph were prepared, two or three of them were fixed immediately. Some of the remaining cultures were observed at frequent intervals while in the microscopic slide incubator. To other cultures of the same series, at intervals of four hours during the first twenty-four hours, neutral red was added. For this purpose the cover slip with its adherent culture was removed from the depression slide, and a drop of neutral red in a concentration of 1:20,000 was added to the culture. The cover slip was replaced and sealed, and after ten minutes of incubation the cultures were studied to determine the reaction of the cells to the neutral red. In several instances, counts were made to determine the number of monocytes present.

Within the first twenty-four hours of incubation, cultures were fixed in formaldehyde bromide after various periods increasing by intervals of from one to three hours. The remaining cultures were fixed after periods increasing by twelve or twenty-four hours.

Before staining, the fixed cultures were washed in tap water for four hours, placed during fifteen to twenty minutes in Delafield hematoxylin solution diluted with 5 parts of distilled water, washed in tap water for thirty minutes, dehydrated in 50 per cent alcohol for five minutes and for the same time in 70 per cent

23. Kindwall, J.: Bull. Johns Hopkins Hosp. **40-41**:39, 1927.

alcohol, in 95 per cent alcohol and in each of two changes of absolute alcohol, then cleared for five minutes in each of two changes of xylene. They were mounted in Canada balsam.

Carbon particles and trypan blue within cells were demonstrable in the cultures stained with paracarmine. Cover slip cultures were fixed in solution of formaldehyde U. S. P. diluted 20 per cent in Ringer's solution for at least one hour, washed in each of two changes of distilled water for five minutes, placed in 70 per cent alcohol for five minutes, stained in Mayer's paracarmine [23a] for from ten to twenty-five minutes, placed for five minutes in 2.5 per cent glacial acetic acid in 70 per cent alcohol, dehydrated for five minutes in 95 per cent alcohol and in two changes of absolute alcohol, cleared for five minutes in two changes of xylene and mounted in Canada balsam.

CELLULAR COMPOSITION OF LYMPH FROM THE THORACIC DUCT

The blood and lymph from nine normal rabbits and seven tuberculous rabbits were examined both in supravitally stained preparations

TABLE 1.—*Differential Cell Counts of Lymph and Blood of Normal Rabbits*

	Differential Count of Lymph				Differential Count of Blood					
		Lymphocytes				Lymphocytes			Polymorphonuclear Leukocytes	
Culture Series	Monocytes	Large	Medium	Small	Monocytes	Large	Medium	Small	Basophilic	Neutrophilic
XXI A	0.1	0.9	7.6	91.4	1.5	1.0	12.0	27.0	1.5	58.0
XXI D	0.2	0.6	8.1	91.1	2.0	2.0	17.0	28.5	1.0	49.5
XXI G	0.4	1.2	9.8	88.6	1.5	0.0	14.0	37.0	2.5	44.5
XXI H	0.4	3.2	10.4	86.0	1.0	2.0	15.0	27.0	10.0	45.0
XXI I	0.6	0.2	6.8	92.4	2.5	0.0	9.0	41.5	2.0	46.0
XXI J	0.2	0.4	6.2	93.2	1.0	0.0	8.0	29.0	0.0	62.0
XXI N	0.3	1.2	7.8	90.7	2.5	1.0	16.0	28.0	1.5	51.0
XXI R	0.0	2.1	8.2	89.7	4.0	2.0	7.0	43.0	7.0	37.0
XXI T	0.6	0.8	5.4	93.7	5.0	0.0	13.0	28.5	0.5	53.0
Average	0.3	1.1	7.8	90.7	2.3	0.9	12.3	32.1	2.8	49.5

and in dry smears stained with Wright-Giemsa solution. In the preparations of the blood, from 200 to 300 leukocytes, and in the lymph preparation, from 500 to 1,500 leukocytes, were counted. Tables 1 and 2 show that the number of monocytes present in the lymph of the thoracic duct was less than 1 per cent in all animals examined with the exception of the lymph from a tuberculous rabbit (table 2, culture series XXI P). It is noteworthy that in each animal with a high blood monocyte count (from 9 to 22.5 per cent) there was no increase in the number of monocytes in the lymph of the thoracic duct. This observation indicates that monocytes of the lymph do not increase with those of the blood and suggests that few enter the blood from the lymphoid tissues and the lymph. It is possible that the animal with a comparatively high monocyte count in the lymph of the thoracic duct had a tuberculous lesion in the proximity of a lymph channel.

23a. The formula is given in Gatenby, J. B., and Cowdry, E. V.: Bolles Lee's Microtomist's Vade-Mecum, Philadelphia, P. Blakiston's Son & Co., 1928, p. 145.

Table 3 gives, for comparison with our own, the differential counts of lymph from the thoracic duct made by several investigators. These figures differ widely.

Kiyono[15] found that 5 per cent of cells in the lymph stored carmine when this substance was administered in large quantity. Bunting and Huston,[24] examining dry smears of lymph from several rabbits, concluded that the lymph of the thoracic duct of the rabbit contained 80 per cent small lymphocytes and 20 per cent larger lymphoid cells but offered no further classification of the larger

TABLE 2.—*Differential Cell Counts of Lymph and Blood of Tuberculous Rabbits*

	Differential Count of Lymph				Differential Count of Blood					
		Lymphocytes				Lymphocytes			Polymorphonuclear Leukocytes	
Culture Series	Monocytes	Large	Medium	Small	Monocytes	Large	Medium	Small	Basophilic	Neutrophilic
XXI O	0.6	0.8	4.0	94.6	12.0	2.0	9.0	11.0	0.0	66.0
XXI U	0.5	1.0	8.6	89.7	22.5	1.0	8.0	38.0	0.0	30.5
XXI V	0.2	2.7	9.5	87.6	18.0	1.0	13.0	32.0	1.0	33.0
XXI W	0.2	0.4	8.2	91.2	11.5	0.0	6.0	39.5	1.5	41.5
XXI P	2.3	1.4	8.1	88.2	18.0	2.0	9.0	44.0	3.0	24.0
XXI Y	0.4	0.9	8.5	90.2	14.0	1.0	10.0	47.0	5.0	27.0
XXI S	0.2	0.4	7.8	91.6	9.0	0.0	9.0	28.0	5.0	49.0
Average	0.6*	1.0	7.8	90.4	15.0	1.0	9.1	34.2	2.2	38.7

* Average of 0.35, excluding series XXI P.

TABLE 3.—*Cellular Composition of Lymph of the Thoracic Duct*

	Small Lymphocytes	"Larger Lymphoid Cells"		Monocytes
Murakami	83.3	16.0		0.7
Bunting and Huston	80.0	20.0		0.0
Murray, Webb and Swan				
Normal rabbit	65.0	30.0		5.0
Rabbit that had received injection of growth of B. monocytogenes	90.0	4.0		6.0
		Medium Lymphocytes	Large Lymphocytes	
Kindwall	88.0	10.0	0.8	0.03
Hall and Furth				
Normal rabbits	90.7	7.8	1.1	0.31
Tuberculous rabbits	90.4	7.8	1.0	0.35*

* In this count series XXI P is excluded.

lymphoid cells. Thorne and Evans[25] found with vital staining almost no monocytes in the lymph of the thoracic duct of the rabbit but did not give the cellular composition of the lymph. They stated that the blood contained many monocytes. Murray, Webb and Swan[26] examined the lymph of two rabbits—one normal and the other given an injection of a growth of B. monocytogenes six days before examination. The former had 6 per cent monocytes in the blood and 5 per cent in the lymph of the thoracic duct; the latter had 45.2 per cent in the blood and

24. Bunting, C. H., and Huston, J.: J. Exper. Med. **33**:593, 1921.

25. Thorne, G. W., and Evans, H. M.: Anat. Rec. **23**:42, 1922.

26. Murray, E. G. D.; Webb, R. A., and Swan, M. B. R.: J. Path. & Bact. **29**:407, 1926.

6 per cent in the lymph. The differential counts of lymph from these rabbits are given in table 3. Kindwall,[23] using supravitally stained preparations of lymph from eight rabbits, enumerates in his table small, medium and large lymphocytes, making a total of approximately 99 per cent lymphocytes in the lymph of the thoracic duct and only 0.03 per cent monocytes (table 3). Simpson[27] wrote ". . . nor with the method of supra-vital staining have monocytes been seen by us in taps of the duct. On the other hand, the lymph in animals stained with Niagara Blue always carries a fair number of unmistakable macrophages. This is easy of explanation for the lymphatic glands are well known seats of the endothelial proliferation which forms macrophage cells and the latter throng both the peripheral and medullary lymph sinuses. It would appear that we cannot have in the lymphatic glands a source of monocyte production and yet this is one of the most active sources of macrophage production." Bloom,[9] after studying the cells of lymph of the thoracic duct from twenty-four rabbits, concluded that the lymph contains lymphocytes of various sizes and almost no monocytes, but he gave no figures. Murakani[28] studied both the peripheral lymph and that of the thoracic duct and found in the lymph of the thoracic duct 16 per cent large lymphocytes and 0.7 per cent monocytes; the remaining cells were small lymphocytes. In the peripheral lymph he found no monocytes.

OBSERVATIONS ON LIVING AND FIXED CULTURES

The study of the tissue cultures of clotted and unclotted lymph from normal and from tuberculous rabbits and from normal dogs yielded similar results.

Immediately After Preparation.—The number of cells in cultures immediately after preparation varied between 10,000 and 200,000. The percentage of large cells, including large and medium-sized lymphocytes and monocytes, varied from 5.4 to 13.8 per cent. Among these, large lymphocytes numbered from 0.2 to 3.2 per cent, medium-sized lymphocytes from 4 to 10.4 per cent and monocytes from 0.1 to 2.3 per cent.

At the Beginning of Incubation.—The monocytes measured from 12 to 15 microns across and had abundant cytoplasm. The nucleus was usually bean shaped or oval and eccentrically situated. The addition of neutral red produced typical rosettes. The lymphocytes took up neutral red in varying amounts in from 4 to 12 scattered vacuoles, but there was no rosette formation. Only an occasional small lymphocyte showed ameboid movement. In the fixed and stained cultures the nuclei of most of the small lymphocytes were slightly indented and each had a thin rim of cytoplasm. This was contrary to the observation of Bergel,[22] who stated that the small lymphocytes acquire indented nuclei after three or four hours of incubation. In the stained smears of lymph of the thoracic duct and in cultures that were fixed immediately after their preparation, mitotic figures were occasionally seen in large lymphocytes.

At from Two to Four Hours.—At the end of two hours there was an increase in the number of ameboid small lymphocytes. In fixed and stained cultures no change in size or arrangement of the nucleus and cytoplasm was evident. Division of some of the large and medium-sized lymphocytes, with the formation of smaller cells, was observed twice in the living cultures kept in a warm stage and watched

27. Simpson, M. E.: J. M. Research **43**:77, 1922.
28. Murakani, J.: Arch. f. exper. Zellforsch. **18**:266, 1936.

under oil immersion. In the fixed cultures mitotic figures were more numerous in the medium-sized and large lymphocytes at the end of four hours than before. Only a few monocytes were observed in the cultures at this time, their numbers depending on the number present at the time of explantation.

At from Four to Six Hours.—There was a further increase in the number of ameboid small lymphocytes. No increase in the number of neutral red staining vacuoles within these cells had occurred. No increase in the size of the living lymphocytes was noted at the end of six hours of incubation. There was, however, a slight increase of the cytoplasm of a few of the small nonmotile lymphocytes, which subsequent observation showed to be dying cells. Mitotic figures were more numerous among the larger lymphocytes than at any other time throughout the life of the cultures. As many as 136 mitotic figures were counted in a single cover slip culture. The cells resulting from the mitosis were smaller than the mother cell. Comparison of differential counts made at this time with those made earlier indicated that there was no transformation of lymphocytes into monocytes. Moreover, transitional forms between lymphocytes and monocytes were not seen.

At from Eight to Twelve Hours.—The number of ameboid small lymphocytes was still increasing, but a small number of the lymphocytes had died, as indicated by absence of ameboid motion, an increase in the amount of cytoplasm and loss of nuclear structure. Several lymphocytes showed fragmentation of nuclei and rupture of the cytoplasmic membrane.

Monocytes were present in about the same numbers as at the time of explantation. Nevertheless, many of them had increased in size. Their cytoplasm was more abundant about the bean-shaped or rounded nucleus than before and contained numerous refractile droplets. When exposed to neutral red, dye accumulated in most of these monocytes, not in the form of rosettes, but in small and large vacuoles throughout the cell, sometimes obscuring the nucleus.

At from Sixteen to Twenty Hours.—At this period the cultures contained the greatest number of ameboid lymphocytes. When they were exposed to neutral red, from 4 to 12 small neutral red stained vacuoles appeared and were scattered throughout the cytoplasm of the lymphocytes. Many of the monocytes had become macrophages and were now more conspicuous, but differential counts made at this time revealed no increase in their number nor were transitional forms between lymphocytes and monocytes found. The number of large and medium-sized lymphocytes had definitely decreased (table 4). Mitotic figures were still seen in these cells.

The cultures to which carbon particles and trypan blue were added at the beginning of incubation showed no increase of phagocytic cells.

From the End of Twenty Hours to the Death of the Cultures.—In the living cultures the number of ameboid lymphocytes had decreased, and at the end of seventy-two hours few were present, but in some cultures ameboid lymphocytes were seen as late as from six to six and a half days after incubation. The number of the medium-sized and large lymphocytes had greatly decreased, and at the end of thirty-six hours none were alive. Large lymphocytes in mitosis were seen after thirty-one hours of incubation in one of the fixed cultures. With the disappearance of the lymphoid cells the monocytes had become more prominent and had taken on the characteristics of macrophages and epithelioid cells. Live macrophages and epithelioid cells were present in the cultures during the twelve-day period of observation. Dunning and Furth [21] observed in similar preparations macrophages in motion over a period of six weeks. The morpho-

logic appearance of the macrophages in our cultures was similar to that in the cultures of Dunning and Furth. An occasional cell had become elongated, but none was transformed into fibroblasts.

In the control cultures made from the buffy coat of the blood under conditions identical with those of the lymph cultures there was abundant growth of monocytes but no transformation of these cells into fibroblasts. In cultures prepared in the same manner from splenic fragments there was, on the contrary, abundant growth of fibroblasts.

REVIEW OF THE OBSERVATIONS

Small Lymphocytes.—The small lymphocytes greatly outnumbered cells of other types present in the cultures and became ameboid within

TABLE 4.—*Differential Cell Counts of Cultures Prepared from Lymph of Normal and Tuberculous Rabbits* *

Source of Lymph	Hours of Incubation	Monocytes and Macrophages	Lymphocytes		
			Small	Medium	Large
Tuberculous rabbit with 2.3 per cent monocytes in lymph	Immediate	2.2	92.4	4.3	1.1
	3	1.8	93.2	3.4	0.8
	5½	0.9	95.9	2.7	0.3
	9½	0.6	96.7	2.3	0.3
	10½	0.9	96.8	1.9	0.2
	21	1.1	97.1	1.6	0.2
Normal rabbit with 0.3 per cent monocytes in lymph-	Immediate	0.30	90.6	7.8	1.2
	2	0.10	90.6	7.6	1.7
	6	0.00	94.7	3.9	1.1
	20	0.10	99.3	0.4	0.2
	50	0.10	99.6	0.3	0.0
Tuberculous rabbit with 0.2 per cent monocytes in lymph	Immediate	0.20	91.2	8.2	0.4
	1	0.30	91.2	8.1	0.4
	7	0.10	96.0	3.2	0.2
	18	0.10	99.5	0.3	0.0

* One thousand cells were counted each time.

one hour after explantation. At the end of from six to eight hours no change in the morphologic characteristics of these cells was noted. Neutral red added to the cultures at different intervals after inoculation showed that the small lymphocytes contained from 4 to 12 neutral red stained vacuoles; the neutral red was not taken up in considerable amounts nor was it collected as rosettes in these cells in the manner characteristic of monocytes. Large numbers of ameboid lymphocytes were present in most cultures at the end of forty-eight hours. Many small lymphocytes were dead or dying at the end of seventy-two hours; nevertheless, a few live lymphocytes persisted as long as six and a half days. Mitotic figures were not seen in small lymphocytes.

Large and Medium-Sized Lymphocytes.—At the time of incubation the number of large lymphocytes varied from 0.2 to 3.2 per cent and that of the medium-sized lymphocytes from 4 to 10.4 per cent. These

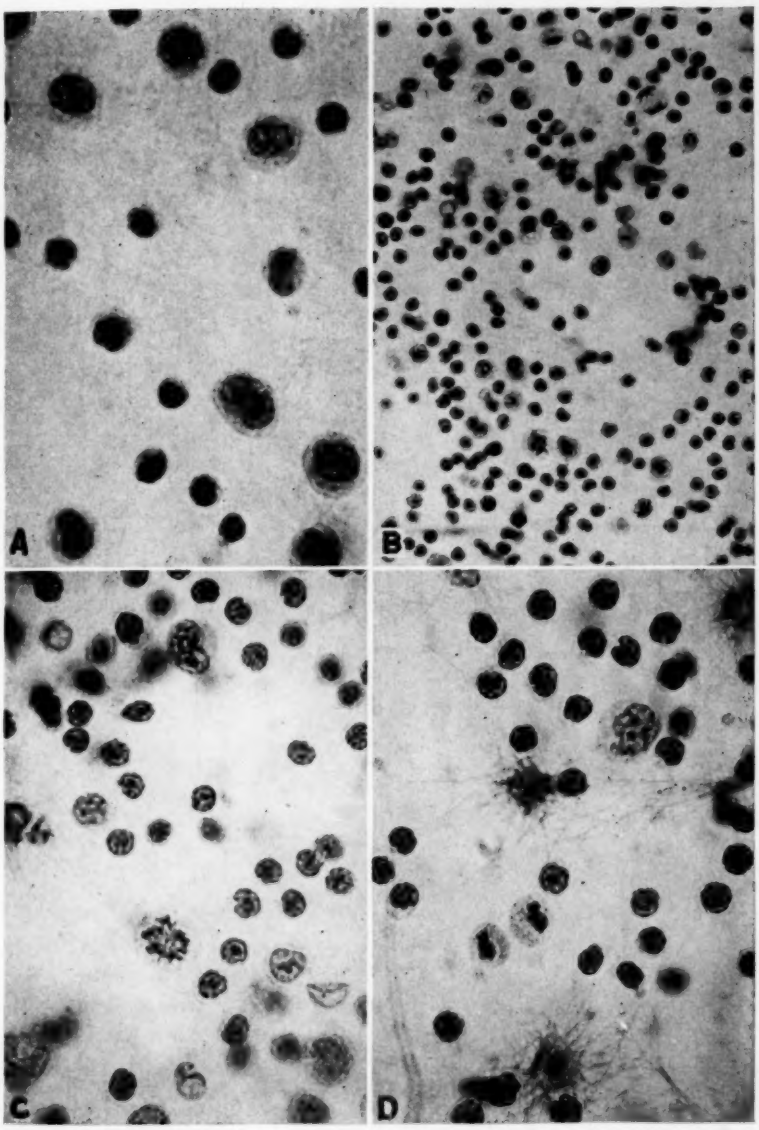

Fig. 1.—Small, medium-sized and large lymphocytes: *A*, in a one hour culture, × 900; *B*, in a five and a half hour culture, × 400; *C*, in a five and a half hour culture, × 900. In *B* and *C* one monocyte is shown, and several of the medium-sized and large lymphocytes contain mitotic figures. *D* shows mitotic division of a large lymphocyte into two medium-sized lymphocytes in a five and one half hour culture; × 900. The cultures were stained with hematoxylin.

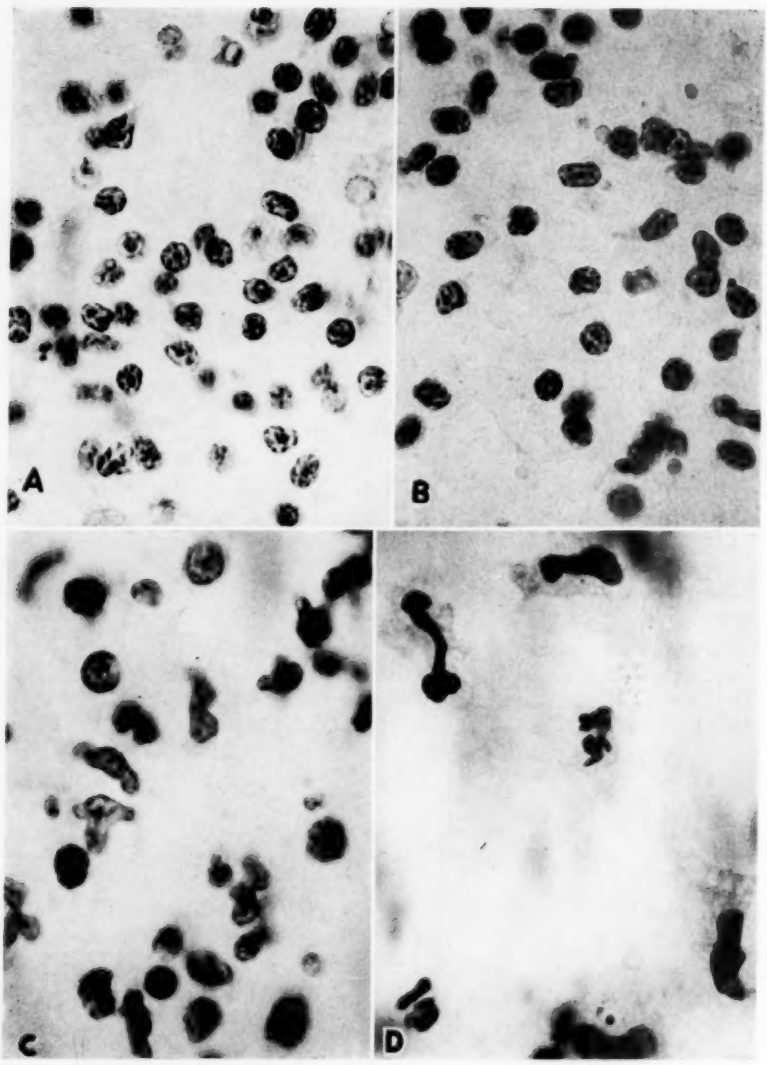

Fig. 2.—*A* shows a twenty-four hour culture in which large lymphocytes are absent; *B*, small lymphocytes in a seventy-two hour culture, several with ameboid outlines; × 900. No monocytes are present. *C* shows ameboid small lymphocytes in a twenty-four hour culture; ×1,300. *D* shows ameboid monocytes in a twenty-four hour culture of the buffy coat of blood. The figure shows the relative abundance of cytoplasm of the monocytes as compared with the lymphocytes; × 1,200.

cells became ameboid after from one to two hours of incubation. After from two to five and a half hours of incubation many mitotic figures were seen among these cells in stained cultures. An occasional mitotic figure was seen in stained smears of fresh lymph from the thoracic duct and in cultures fixed after one hour of incubation. One mitotic figure was seen in a culture stained after thirty-one hours of incubation. Division of large and medium-sized lymphocytes into smaller cells was observed in the living cultures. At the end of twelve hours the number of larger lymphocytes had decreased so that at the end of twenty-four hours very few living large lymphocytes were present. The disappearance of large and medium-sized lymphocytes from the cultures is due, we believe, to the division of these cells into smaller lymphocytes. Transformation of these cells into monocytes was not observed.

Monocytes.—Monocytes were present in the lymph at the time of explantation, their number varying in different animals from 0.1 to 2.3 per cent. Excluding the lymph of a tuberculous animal containing 2.3 per cent monocytes, the average monocyte count of the lymph was 0.33 per cent. The fate of the monocytes was followed in cultures to which neutral red and trypan blue were added. Counts of the total number of monocytes present in the cultures were made at different intervals. The number of monocytes present at the end of twelve to twenty-four hours never exceeded the number at the time of explantation. By the end of eight to twelve hours the monocytes were transformed into macrophages, and at the end of forty-eight hours many of these cells had the appearance of epithelioid cells.

The lymph used in series XXI H had at the time of explantation approximately 800 (0.4 per cent) monocytes, and at the end of forty-eight hours of incubation, approximately 450 macrophages (0.25 per cent) in each culture, but no monocytes.

COMMENT

The experiments described do not support the opinion of Bloom and other workers that lymphocytes of the thoracic duct are transformed in vitro into monocytes.

Since monocytes were present in only small numbers in our cultures of the lymph of the thoracic duct, and fibroblasts were absent, it was desirable to determine whether the medium used was suitable for multiplication of monocytes and fibroblasts. In five experiments the buffy coat of the blood, and in seven experiments the spleen, of the rabbit whose lymph was cultured were incubated in vitro in the medium that was used for the lymph cultures. The observations on these cultures will not be described in detail. From the fragments of the buffy coat monocytes in great numbers migrated into the explant within twenty-

four hours. In the splenic cultures fibroblasts and histiocytes grew in large numbers. These observations indicate that the medium used in the lymph cultures is suitable for the growth of fibroblasts. Moreover, monocytes appeared in the lymph cultures in variable numbers depending on the number present before incubation. The great number of large lymphocytes in the lymph is noteworthy in this connection, for in fixed and stained preparations of tissue these cells can be distinguished from monocytes with difficulty. Moreover, in the degenerating lymphocyte, the nucleus becomes homogeneous, the cytoplasm more voluminous, and the cell assumes an appearance similar to that of a blood monocyte.

Bloom collected lymph by drawing it up, by means of a small pipet, from an incision made into the thoracic duct. He quoted Kindwall,[23] who found that the lymph collected in the hollows of the muscles and fascia contained 90 per cent lymphocytes. Yet it is possible that an occasional endothelial cell or fibroblast is drawn up into the pipet. The percentages of monocytes in the lymph of the thoracic duct used for incubation are not given by Bloom. It is possible that the various types of peritonitis produced experimentally in his rabbits had increased the number of monocytes in the lymph of the thoracic duct.

A possible source of fibroblasts in tissue cultures is the organ extract used to promote growth. No mention is made in the articles of the workers quoted that the extract was examined to ascertain the absence of cells. In our experience centrifugation of cut up tissue at the usual high speed during ten minutes does not render the supernatant fluid entirely cell free.

Bloom[9] did not give photomicrographs but illustrated selected cells by drawings. He stated that "after a few days some of the polyblasts change in size and shape and become typical fibroblasts." His figure 4, table 24, given to illustrate a fibroblast in mitosis, might be an epithelioid monocyte as well as a fibroblast. Dunning and Furth[21] found viable macrophages and epithelioid cells in tissue cultures after six weeks of incubation, but they did not see transformation of these cells into fibroblasts.

Bloom found that the smaller lymphocytes had developed into larger ones and observed very few large lymphocytes in the early cultures. In our experience large and medium-sized lymphocytes made up from 5 to 12 per cent of the cells of the lymph. Mitotic figures were seen in these cells as late as thirty-one hours after incubation, and in some of the fixed cultures there were as many as from 100 to 136 mitotic figures. In the living cultures after from three to five hours of incubation we observed the division of large lymphocytes into smaller lymphocytes.

Bergel[22] is quoted in confirmation of the observations of Bloom. He cultured small pieces of the mesenteric lymph nodes of rabbits and lymph from the efferent lymphatics of the mesenteric lymph nodes. He stated that the young lymphocytes stain so intensely that the nucleus, which is large, and the cytoplasm, which is small in amount, cannot be distinguished one from the other. The dark staining nucleus acquires a distinctive structure in the course of further growth at a time when the quantity of cytoplasm is increasing. The nucleus becomes indented and eccentrically located. In cultures observed at from two to seven days he found transitional forms between lymphocytes and monocytes, whereas Bloom maintained that the transformation takes place within twenty-four hours.

Bergel's statement that small or young lymphocytes in vitro do not show the usual cellular structure is doubtless erroneous. In overstained preparations, such as those shown in his figures, living and dead cells are indistinguishable. Most of the cells shown in his pictures do not have the appearance that monocytes assume in vitro. In the cells illustrated in his photomicrographs no nuclear structure is evident; the cells have the appearance of degenerated lymphocytes.

The statement is often made that lymph contains almost no monocytes. In our experience the percentage of monocytes varied from 0.14 to 0.6 per cent (one tuberculous rabbit having 2.3 per cent), so that a cover slip preparation containing 10,000 to 200,000 cells had from 14 to 1,200 monocytes.

SUMMARY

The lymph of the thoracic duct of the rabbit contains medium-sized and large lymphocytes in large numbers (from 5 to 13 per cent) and an occasional monocyte (from 0.1 to 2.3 per cent, averaging 0.45 per cent), the remaining cells being small lymphocytes.

The number of monocytes in the lymph of the thoracic duct is not conspicuously increased in tuberculous rabbits, even though there are from 9 to 22.5 per cent monocytes in the circulating blood.

The small lymphocytes of the lymph of the thoracic duct exhibit ameboid motion when incubated in tissue cultures in vitro, and some remain alive for from two to six days, but most of them die within forty-eight hours.

The large lymphocytes divide by mitosis into smaller lymphocytes during the first thirty-one hours of incubation. The greater number of divisions occur after from three to five hours of incubation.

Monocytes present in the lymph become actively phagocytic, and after forty-eight hours these macrophages assume the characteristics of epithelioid cells.

Evidence is wanting that in tissue cultures lymphocytes are transformed into monocytes and monocytes into fibroblasts. Tissue culture studies support the view that these cells represent independent cell types.

Case Reports

METASTASIZING FIBROMYOMA OF THE PLEURA

Report of Two Cases

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The formation of metastases in distant organs by apparently benign neoplasms has been the subject of considerable controversy. In particular, the so-called metastasizing myoma has repeatedly been said to differ from the common myoma by its cellular irregularity, the greater chromatin content of its nuclei and the presence of mitoses. Ewing [1] referred to a morphologic change in the tumor cells. The cells are shorter and more rounded. Giant cells appear, the intercellular connective tissue becomes scanty or disappears, and the walls of the blood vessels are defective. He comes to the conclusion that, as far as he has been able to learn, no case has been fully studied in which definite variation from the usual structure of leiomyoma has been wanting, although in some instances this variation has been very slight. This type of tumor has been observed in the uterus and the gastro-intestinal tract. In his discussion of pulmonary metastases from benign fibromyomas of the uterus, R. Meyer [2] pointed out that it is difficult to understand how small pieces could break loose from a firm myoma to enter the blood stream. He also considered the possibility that the myomatous nodes found in the lungs might be independent of the uterine neoplasms and might represent congenital malformations.

I have observed two cases of tumor of the pleura which are of great interest for several reasons and deserve detailed description. In both instances the tumor had originated from the parietal pleura, and in both instances it was composed of connective tissue and smooth muscle fibers. Fibromyoma of the pleura is extremely rare. Furthermore, in both cases regional lymph nodes were the site of blastomatous changes which were morphologically identical with those of the pleura and impressed one as metastases. Histologically, an extensive study of the pleural tumors did not reveal any of the standard criteria of malignancy. In one instance, both the primary tumor and the apparent metastases contained xanthomatous areas. Both tumors occurred in women; one was an incidental finding; the other caused death by interfering with the venous circulation.

CASE 1

History.—A white girl of 17 years and of German-American parentage had for five months experienced progressive enlargement of the abdomen, associated

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† Dr. Jaffé died on Dec. 17, 1937.

1. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928.

2. Meyer, R.: Die pathologische Anatomie der Gebärmutter, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 8, pt. 1.

with occasional nosebleeds. During the last two of the five months she had also noticed puffiness of the feet and ankles. She had had a single menstrual period when she was 14 years old. The following year she had two menstrual periods, the second one lasting five days. Since that time no menstruation had occurred. However, she had regular monthly pains in both lower quadrants of the abdomen.

The increase in size of the abdomen was not associated with pain. There were gradually increasing drowsiness and a tired feeling. She had had pneumonia in childhood and an appendectomy five years ago.

The outstanding physical findings were marked dilatation of the veins of the chest and abdomen, distention of the abdomen, with evidences of much free fluid, marked enlargement of the liver and spleen and presence of a firm nodular mass in the right epigastrium. The distention of the abdomen caused flaring of the lower ribs, and the lower half of the right side of the chest was flat, the flattening extending posteriorly to the posterior axillary line. In this region the breath sounds were absent. The heart was somewhat displaced to the left. A double murmur was audible over the precordium, and there was a musical murmur to the right of the heart. The pulse rate was 120, the blood pressure 120 systolic and 74 diastolic, and the respiratory rate 24.

Repeated roentgenologic examinations of the chest disclosed a well defined area of marked opacity in the anterior and median portion of the right side of the chest, extending upward into the infraclavicular region. A roentgenologic picture made after a pneumoperitoneum showed several nodes with evidence of much calcification in the right epigastrium, and there was also calcification of the blood vessels of the pelvis and of the lower extremities.

Laboratory Findings: The erythrocyte count was around 3,000,000, and the hemoglobin content was between 47 and 55 per cent. The white count was 8,700, with 87 per cent neutrophils, 10 per cent lymphocytes and 3 per cent monocytes. The reticulocyte count was 1 per cent. Urinalysis, the electrocardiogram and microscopic and bacteriologic examinations of the abdominal fluid did not yield significant results. The Wassermann and Kahn tests were negative. The calcium content of the plasma was 9.20 mg. per hundred cubic centimeters, and the icterus index was 4.85. The Takata-Ara test with the serum was strongly positive.

The final diagnosis was that of a malignant tumor of the mediastinum or chest with metastases to the abdomen. The possibility of calcification of tuberculous lymph nodes of the abdomen was also considered.

The patient's abdomen was repeatedly tapped, and each time several liters of a clear straw-colored fluid with a specific gravity of about 1.004 was removed. The patient lingered in the hospital, gradually wasting away. Toward the end her temperature rose to 103 F., and she died seven months after admission and approximately one year after the onset of her symptoms.

Necropsy.—At the time of her death the patient was much emaciated. That her weight amounted to 63 Kg. was mainly due to the extensive subcutaneous edema, which involved the entire body except the face, neck and upper extremities. The abdomen was distended, and the veins over the anterior wall of the chest and abdomen were prominent and tortuous. The abdominal cavity contained 10,000 cc. of a clear straw-colored fluid, and the greater omentum was adherent to the anterior abdominal wall and to the loops of the small intestine. The liver was displaced 17 cm. below the costal arch, and the lower pole of the spleen was found 1 fingerbreadth below the costal arch, in the anterior axillary line.

Originating from the median portion of the right side of the diaphragm, obscuring the angle between the diaphragm and the pericardial sac and involving the greater part of the right lateral wall of the pericardial sac, a large, roughly

ovoid firm mass was found protruding into the right pleural cavity and into the pericardial sac. The free surface of the mass was covered by the parietal pleura, which was smooth and shiny save for circumscribed areas in which the much compressed lower pulmonary lobe was attached by thin fibrous strands. The mass measured 18 by 15 by 10 cm. in diameter and was composed of a dense and fascicular tissue, the color of which varied from light purple-tan to pale gray. Inserted into this tissue were parallel light yellow-gray lines of stony consistency, and in the posterior portions of the mass the lines fused together to form a solid, hard area which required sawing in order to expose its structure. Except for the fibrous strands referred to, the mass was well separated from the lung. The middle and lower pulmonary lobes were compressed and airless, while the upper lobe contained a small amount of air. Loose fibrous strands also fixed the pericardial sac to the surface of the heart. Where the mass protruded into the pericardial sac, its free surface was slightly lobular. The intrapericardial portion of the inferior vena cava was much compressed, and when the heart was opened the ostium of the inferior vena cava was found to be completely occluded by the extrinsic compression. However, the wall of the vein was not invaded, and the intima was everywhere smooth and pale.

In the lower half of the posterior mediastinum, to the left of the thoracic portion of the aorta and partially overlapping it, a group of firm spherical or ovoid nodes were found. The nodes measured from 2 to 7 cm. in greatest diameter. Some of them fused with the pleural mass, while others were well separated from it. They consisted of a tissue the texture of which was identical with that of the main mass. These nodes compressed the supradiaphragmatic portion of the esophagus, which admitted the tip of the little finger with difficulty.

About the cardia of the stomach and along the lesser curvature, also at the hilus of the liver and that of the spleen and about the head of the pancreas there were numerous slightly lobular nodes which were stony hard and averaged 6 cm. in diameter. The surfaces of these nodes were smooth, and loose fibrous bands connected them with the adjacent organs, particularly with the stomach. When sawed apart the nodes were found to be composed of a bonelike light yellow tissue that was interspersed with islands of a softer, light purple-tan tissue. The stomach was wedged in between the stony masses which, except for the compression, did not affect its structure, the mucosa being intact and pale, with a purple-gray color.

The weight of the heart was 150 Gm.; that of the liver, 1,000 Gm. The capsule of the liver was thickened, and when this organ was sectioned the lobular markings were found to be prominent, and there was also a marked increase of the periportal tissue. The spleen measured 20 by 12 by 6 cm. It was firm and much congested with distinct trabeculae. The right adrenal was surrounded by indurated fat tissue with many stony inclusions. The left adrenal weighed 5 Gm. The kidneys weighed 410 Gm. and were moderately congested. The pancreas, intestine, internal genitalia and left lung did not present any abnormalities. The veins over the inferior aspect of the right half of the diaphragm were much dilated. The intima of the inferior vena cava and of the common and external iliac veins showed hyaline plaques, and in the iliac veins there were also many calcified plaques. There was diffuse calcification of the walls of the medium-sized and small blood vessels of the thighs, and when the finger was passed over incisions made through the muscles of the thighs, the small calcified vessels felt like the bristles of a stiff brush.

Microscopic Examination.—Sections taken from the large mass of the right pleural cavity revealed that it had originated from the thickened pleural cover of

the diaphragm, while the diaphragm itself was practically unchanged (fig. 1*A*). The mass consisted chiefly of a dense fibrillar connective tissue, which was in continuity with the superficial connective tissue layer of the diaphragm. The cellularity and vascularity of the connective tissue varied in different areas, but in most places the tissue was loosely infiltrated by round cells, which were slightly larger than lymphocytes and had compact nuclei and narrow rims of cytoplasm. The infiltrations were most marked near the diaphragm, where the cells sometimes arranged themselves in rows along the blood vessels and between the collagenous bundles. In the cellular areas one found regular spindle-shaped fibrocytes encased in a net of Van Gieson red ground substance. The tissue often formed interlacing bundles. In sections stained with azocarmine-aniline blue-orange G (Heidenhain-Mallory) single smooth muscle fibers could be recognized between the connective tissue fibers. They were very long and showed fine longitudinal fibrillation, and their nuclei had rounded poles and a coarser and more sharply defined chromatin net than the nuclei of the fibrocytes. Here and there a bundle of smooth muscle fibers was seen traversing the connective tissue (fig. 1 *B*).

In the less cellular areas the collagenous ground substance predominated and showed a tendency to undergo hyalinization. In the cellular areas single small spherical concretions of calcium were found, and these concretions increased in number and size as the ground substance increased. Some of the concretions were derived from the intercellular tissue; others could be traced to the blood vessels, the walls of which became impregnated with lime salts. Later the lumens were obliterated by intimal proliferation, and the connective tissue filling the lumens underwent calcification. In the areas with abundant hyalinized ground substance, the calcification was diffuse, and there were also variously shaped spicules of calcified bone.

In the part of the tumor that protruded into the pericardial sac, near the pericardial surface, there were glandlike tubular structures which were lined by a single layer of pale-stained cuboid cells, the inner portion of which formed a convex cap that bulged into the empty lumen.

The isolated masses in the posterior mediastinum and the upper part of the abdomen proved to be derived from lymph nodes. The enlarged lymph nodes retained their shape and also remained separated from one another. The lymphatic tissue had been replaced by dense fibrillar connective tissue that fused with the capsule and the trabeculae, obscuring the sinuses and reducing the secondary lymphocytic nodules of the cortex to isolated small accumulations of lymphatic cells (fig. 2). The connective tissue was vascular and often formed onion peel-like layers about the vessels. Besides fibrocytes, fixed and free histiocytes and mast cells, the connective tissue contained single long smooth muscle fibers (fig. 3). The residues of lymphatic tissue were composed either of small and medium-sized lymphocytes or of lymphocytes and plasma cells. Some of the islands of lymphatic tissue contained iron pigment, while others revealed a transformation of the reticulum cells into large lipoid-filled cells. Some of the medium-sized arteries showed calcification of the media and fibrous thickening of the intima.

In the lymph nodes in which the replacement by connective tissue had not yet reached the advanced stage, the fibrillar tissue was seen growing into the nodes from the hilus along and inside the sinuses, and also the marginal sinus became obliterated by an ingrowth of connective tissue. In this connective tissue single smooth muscle fibers could be clearly recognized. Deprived of their connection with the sinuses the medullary cords shrank and disappeared, and the secondary nodules of the cortex became surrounded by connective tissue, which encroached on them and gradually replaced them.

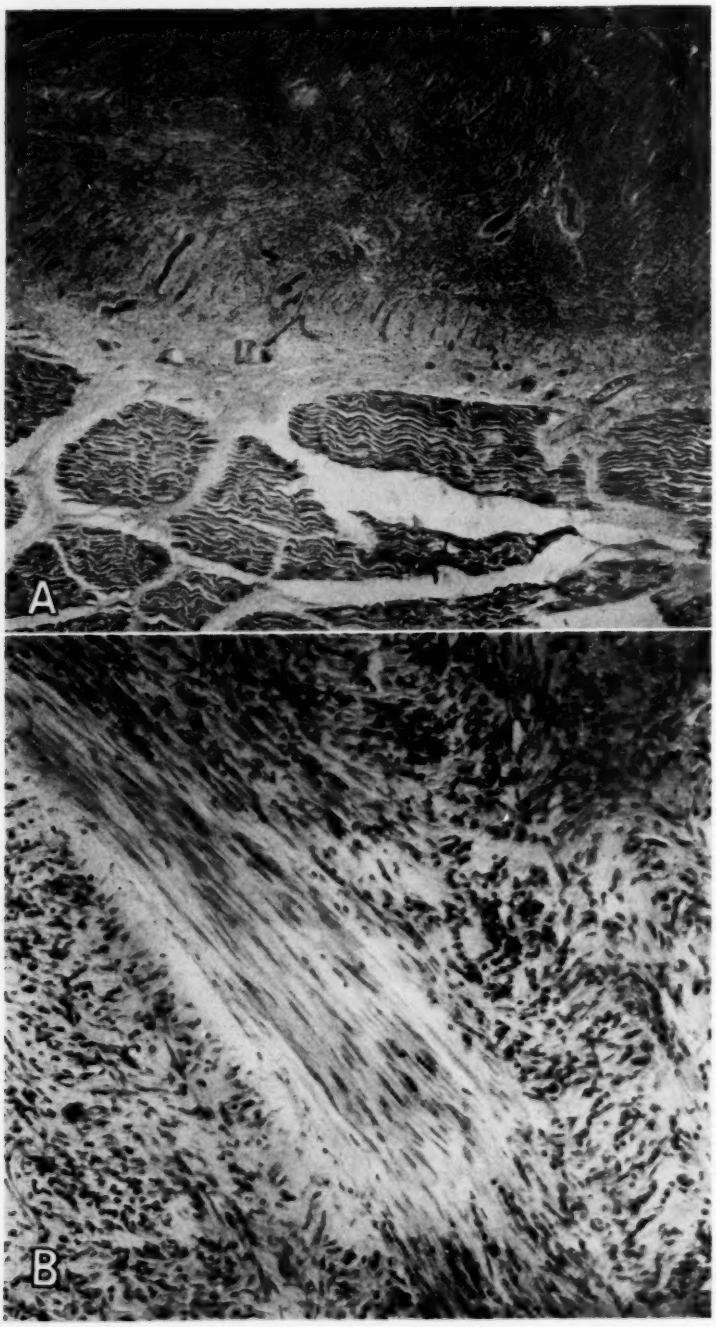


Fig. 1 (case 1).—*A*, origin of the tumor from the diaphragmatic pleura; hemalum, eosin; × 20 (reduced). *B*, bundle of smooth muscle fibers in the pleural tumor; hemalum, eosin; × 150 (reduced).

Fig. 2 (case 1).—Metastasis to the posterior mediastinal lymph node; azocarmine stain; × 6.5 (reduced).

Fig. 3 (case 1).—Metastasis to a posterior mediastinal lymph node. A long smooth muscle fiber crosses the field, surrounded by loose connective tissue; azocarmine stain; × 600.

The stony consistency of the lymph nodes of the upper part of the abdomen was due to extensive calcification of the hyalinized connective tissue which had replaced the lymphatic tissue. The calcified hyalinized trabeculae fused to form solid areas, and there was also an occasional spicule of bone inserted into the calcified matter. Some of the deposits of calcium were found to be derived from blood vessels. Everywhere one found in the midst of the calcified tissue islands of cellular connective tissue with muscle fibers.

The right adrenal was surrounded by a dense connective tissue that fused with the thickened capsule. This connective tissue contained numerous deposits of lime salts. From the capsule strands of connective tissue extended into the cortex, and in the inner part of the fascicular zone the capillaries were often surrounded by a layer of hyaline connective tissue, which compressed the adjacent cortical cells. The lipoid content of the cortical cells was diminished.

The spleen showed marked passive congestion with diffuse thickening of the fibrillar reticulum, and there were also small siderofibrotic and siderocalcific intratrabecular nodules. About some of the larger intratrabecular veins thick coats of calcium salts were found. There was one small subcapsular area of necrosis of the pulp with deposits of hematoidin.

The liver presented the microscopic picture of congestive cirrhosis, and also the kidneys were much congested. The renal arteries and arterioles were unchanged.

The intima of the iliac veins showed many circumscribed fibrotic plaques, which protruded into the lumen and contained compact deposits of lime salts. Underneath the calcified plaques the media was much atrophied. Many of the veins in the muscles of the thighs revealed similar thickening and calcification of the intima with atrophy of the media.

Summary of Anatomic Findings.—The findings were: a large fibromyoma of the pleural cover of the median portion of the right half of the diaphragm, extending into the pericardial sac and obscuring the right diaphragmatic-pericardial angle; confluent and focal areas of calcification, especially in the posterior portions of the tumor; marked compression of the right lung and occlusion of the supradiaphragmatic portion of the inferior vena cava by the tumor; metastases to the posterior mediastinal, peribiliary, peripancreatic, perigastric and splenic hilar lymph nodes, with extensive calcification and ossification; compression of the esophagus by the metastases to the posterior mediastinal lymph nodes; congestive cirrhosis of the liver; chronic passive congestion of the spleen and kidneys; sclerosis of the inferior vena cava, iliac veins and veins of the thighs; marked ascites; caput medusae; marked generalized anasarca; induration and calcification of the periadrenal tissue of the right side; slight atrophy of the heart; moderate compensatory emphysema of the left lung.

CASE 2

History.—A white woman aged 56 years was brought to the hospital in a moribund condition. Her daughter stated that three months ago a physician told her that she had a cancer of the uterus. Apparently nothing was done about her condition. While working, on a hot day, she collapsed and became pulseless and cyanotic. She died two hours after admission.

Necropsy.—As the essential anatomic feature, the autopsy disclosed an adenocarcinoma of the corpus uteri with extensive metastases to the mesentery, the greater omentum, both ovaries and to a polyp of the cervix uteri. There were marked ascites and multiple intramural fibromyomas of the uterus, simple serous cysts of the left ovary, diverticula of the descending and of the sigmoid colon and

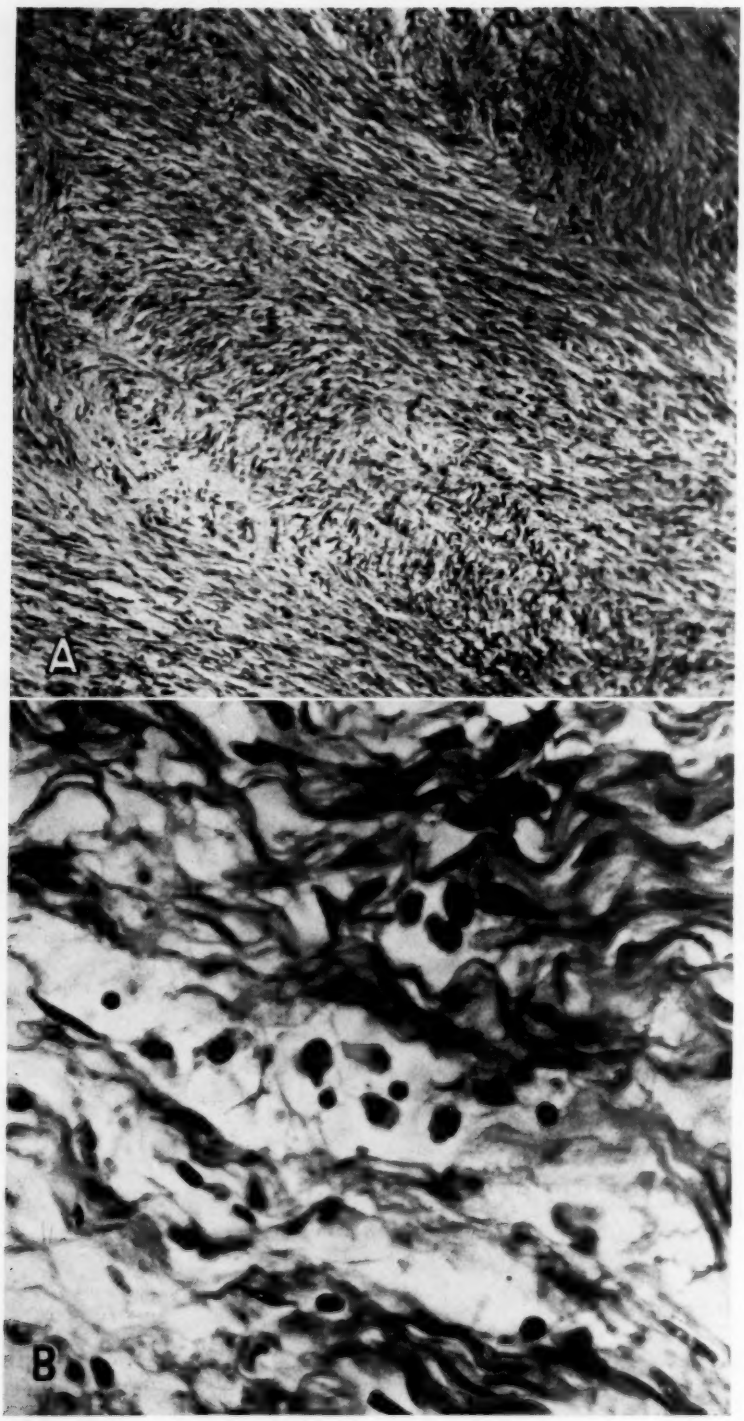
a nodose goiter with ancient resection of the right lobe. The woman was obese, weighing 80 Kg. When the right pleural cavity was examined, the lower pulmonary lobe was found to be much compressed and displaced forward and slightly downward by a firm spherical mass, which seemed to originate from the posterior thoracic wall between the seventh and ninth ribs. Medially it extended to the lateral aspect of the eighth and ninth dorsal vertebral bodies, to which it was adherent. The surface was covered by thickened parietal pleura, the veins of which were much distended. The mass measured 13 by 12.5 by 12 cm. in diameter and was loosely fixed to the posterior aspect of the right lower pulmonary lobe. On being sectioned, it presented a fascicular structure and was light grayish tan. Scattered throughout the mass but most numerous in the central portions, there were discrete and confluent irregular areas of a bright light yellow-gray color, measuring up to 15 mm. in diameter.

Below the hiatus of the diaphragm and about the upper abdominal portion of the aorta there were groups of enlarged lymph nodes the gross appearance of which was so striking and resembled so closely that of the pleural tumor that the possibility of metastases from the uterine carcinoma could be discarded at first glance. These nodes were moderately firm, somewhat rounded and well separated from each other. The capsule was stretched but intact, and the greatest diameter varied from 20 to 30 mm. The sectioned surfaces were pale, gray-tan, fascicular and interspersed with light yellow-gray areas, up to 10 mm. in diameter.

Microscopic Examination.—The free surface of the pleural tumor was covered by a thick capsule of dense connective tissue, which was vascular and near the surface contained single glandlike tubular structures, which were lined with a regular cuboidal epithelium. There were focal dense accumulations of small lymphocytes. From the inside of the capsule strands of connective tissue radiated into the tumor. The tumor itself was composed of interlacing bundles of long slender cells (fig. 4*A*) that stained brass-yellow after the Van Gieson method and red-brown with the Heidenhain-Mallory azocarmine method. The cells, which revealed a delicate longitudinal fibrillation, possessed regular oval nuclei with rounded ends and dense chromatin granules. After long searching a single mitosis with regular chromosomes could be found. With the azocarmine stain fine blue fibrils could be demonstrated between the cells, and in places the interstitial tissue became more abundant and assumed a hyaline appearance. There was a moderate number of blood vessels, some of which had thickened and hyalinized walls.

In circumscribed areas the tumor tissue was loosened, the long slender cells were spread apart, and in the empty spaces between the fibers round cells were found, which were larger than lymphocytes and had round or oval nuclei and distinct rims of homogeneous cytoplasm (fig. 4*B*). With increase in size, small lipoid droplets became visible in the cytoplasm, and in the largest of the cells fat droplets filled the entire cell body so that only thin bridges of cytoplasm were left (fig. 5*A*). Occasionally the fine bridges had broken, and a single large drop of anisotropic fat occupied the cell. The vacuolated cells with deeply stained nuclei sometimes piled up to form a solid nest with a small blood vessel in the center.

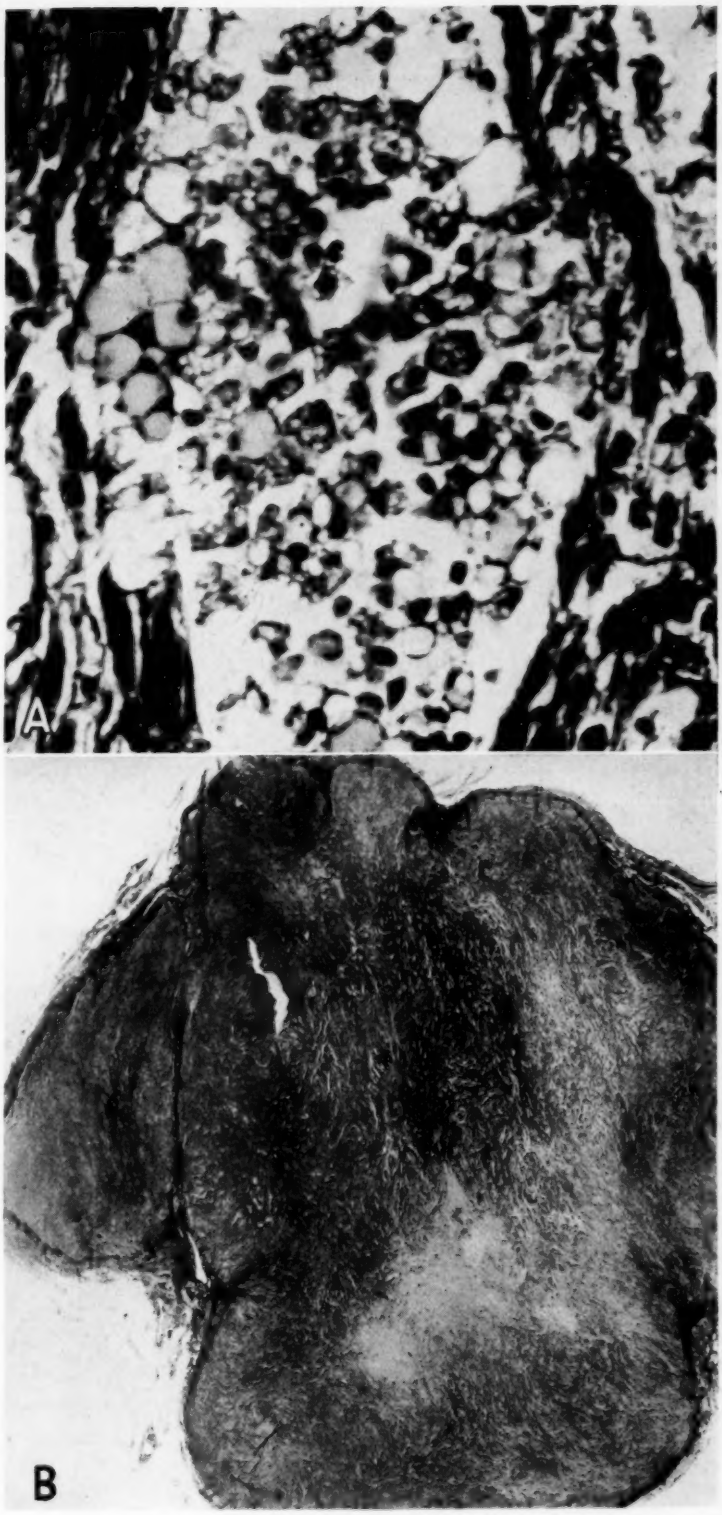
Microscopically, the retroperitoneal lymph nodes of the upper part of the abdomen closely resembled the pleural tumor. The nodes were made up of interlacing bundles of long slender cells (fig. 5*B*), and there were many circumscribed interstitial accumulations of cells with vacuolated cytoplasm. The thickened capsule of the node fused with the scanty stroma between the long cells. In the periphery, underneath the capsule, one saw an occasional small group of lympho-



EXPLANATION OF FIGURE 4

A, tumor of the pleura (case 2); hemalum, eosin; × 150 (reduced).

B, an area of interstitial edema with free histiocytes in the tumor of the pleura; hemalum, eosin; × 600 (reduced).



EXPLANATION OF FIGURE 5

A, tumor of the pleura (case 2), showing a group of xanthoma cells between the smooth muscle fibers; azocarmine; × 600 (reduced).

B, metastasis in a periaortic lymph node; azocarmine stain; × 5 (reduced).

cytes, representing the residue of a secondary nodule of the cortex. In the fat tissue about the nodes, bordering the capsule, there were many infiltrations of small lymphocytes.

On the basis of the microscopic observations the pleural tumor was diagnosed as leiomyofibroma with xanthomatous areas, and the enlarged lymph nodes below the diaphragm were interpreted as metastases of this tumor.

COMMENT

The fibroma is the most common benign tumor to be found on the parietal or visceral pleura. In some cases, it has been linked to nerves and has been designated as neurofibroma, perineural fibroblastoma or myxoneurofibroma.[3] The pleural neurofibroma may be single or it may be a part of generalized neurofibromatosis. In other cases, pleural fibroma has seemed to originate from the submesothelial connective tissue.[4] Russow [5] traced the osteoid fibroma of a boy aged 6 years to the connective tissue of the bone marrow of a rib. In the cases observed by Foltz [4b] and Hollmann [4c] the tumor was attached to the diaphragm. In addition to fibroma, angioma, fibrolipoma and lipoma of the pleura have been described.[6] In 1918, I observed a cellular leiomyoma of the visceral pleura of the left upper pulmonary lobe, a description of which was published by Kornitzer.[7] Jacobaeus and Key,[8] in their paper on intrathoracic tumors, referred to an edematous fibromyoma in a man 23 years of age and to an edematous fibroma with strands of smooth muscle fibers in a man aged 28. In both cases the histologic diagnosis was made by Hedrén. Wessén [9] described in a woman 28 years of age a retropleural tumor which protruded from an intercostal space into the pleural cavity and was removed surgically. Microscopically, this growth was composed of a fibrous ground substance with many bundles of smooth muscle fibers and groups of xanthomatous cells. The author called the tumor a "cellular xantholeiomyoma." It may be mentioned

3. Andrus, W. De Witt: J. Thoracic Surg. **6**:381, 1937. Banse, J.: Ueber intrathoracische Fibrome, Neurome und Fibrosarkome, Inaug. Dissert., Greifswald, H. Adler, 1908. Gery, L.: Bull. et mém. Soc. anat. de Paris **93**:821, 1923. Grawitz, P.: Deutsche med. Wchnschr. **34**:1123, 1908. Harrington, S. W.: J. Thoracic Surg. **3**:590, 1934. Keller, W. L., and Callender, G. R.: Ann. Surg. **92**:666, 1930.

4. (*a*) Fischer, W.: Die Gewächse der Lunge und des Brustfelles, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1931, vol. 3, pt. 2. (*b*) Foltz, P.: Arch. ital. di anat. e istol. pat. **4**:84, 1933. (*c*) Hollmann, W.: Med. Klin. **32**:1585, 1936. (*d*) Mintz, N.: J. Mt. Sinai Hosp. **2**:38, 1935. (*e*) Schmidt, J. E. W.: Ueber Fibrome der Lungenpleura, Inaug. Dissert., Greifswald, H. Adler, 1903. (*f*) Unger, R.: Zentralbl. f. d. ges. Tuberk.-Forsch. **37**:1, 1932.

5. Russow, A.: Jahrb. f. Kinderh. **53**:340, 1931.

6. (*a*) Garre, C.: Deutsche med. Wchnschr. **44**:617, 1918. (*b*) Klemperer, P., and Rabin, C. G.: Arch. Path. **11**:385, 1931. (*c*) Von Rokitanski, K.: Lehrbuch der pathologischen Anatomie, ed. 3, Vienna, W. Braumüller, 1881, vol. 3.

7. Kornitzer, E.: Berl. klin. Wchnschr. **56**:1039, 1919.

8. Jacobaeus, H. C., and Key, E.: Acta chir. Scandinav. **53**:573, 1921.

9. Wessén, N.: Acta chir. Scandinav. **53**:621, 1921.

here that leiomyoma has also been observed in the lung.[10] The tumor reported by Kunz [11] and one of the multiple tumors reported by Deussing [10b] contained glandular structures. In a discussion of Harrington's paper on thoracic neurofibromas Phillipps quoted from reports on several cases of xanthomatous tumors of the pleura.

The literature contains many reports on sarcoma of the pleura, of which different types have been described. Much interest has been devoted to the "giant pleural sarcoma" which, in spite of its huge size, is relatively benign, especially so far as it does not tend to produce metastases. This relative benignity offers good chances for successful surgical removal. In the case which Mallory [12] demonstrated the pleural tumor weighed about 20 pounds (9 Kg.). Histologically, such a tumor is either a spindle cell sarcoma or a fibrosarcoma, and in the latter instance the evidences of malignancy may be slight and may be confined to circumscribed areas of increased cellularity and nuclear pleomorphism or focal invasion of the capsule. Catron [13] reported on a large leiomyosarcoma that was incidentally found attached to the left lung of a woman aged 83 years, who had died from coronary sclerosis. It is remarkable that many of the pleural giant sarcomas did not cause any serious clinical manifestations and were incidentally found either at autopsy or during routine roentgen examination of the chest. In some cases, however, they were associated with severe dyspnea, cyanosis, failure of the right side of the heart, clubbing of the fingers and pulmonary osteo-arthropathy. Giant sarcomas of the pleura have been described by Eggers,[14] Schneider,[15] Klemperer and Rabin [6b] (reviewing the literature), Lichtenstein,[16] Lyssunkin [17] Neumann,[18] Nevinny,[19] Sala [20] and many others. In the cases reported by Schneider,[15] Sala [20] and Palasse and Roubier,[21] the tumor had apparently developed from the pleural cover of the diaphragm. Klemperer and Rabin [6b] stated that a sarcoma derived from the thoracic wall has a greater tendency to metastasize than a sarcoma of the visceral pleura.

In the first of my two cases of pleural tumor the connective tissue took active part in forming the tumor. In many places it predominated, and the muscle fibers were confined to isolated bundles. From other areas, muscle fibers were completely absent. One is therefore justified in speaking of a mixed tumor. The connective tissue was mature, and although it was infiltrated by round cells, it did not have the appearance of embryonic connective tissue as described by Klemperer

10. (*a*) Forkel, W.: Ztschr. f. Krebsforsch. **8**:390, 1909. (*b*) Deussing, R.: Multiple primäre Myome der Lunge, Inaug. Dissert., Munich, R. Müller & Steinicke, 1912. (*c*) Franco, E. E.: Tumori **15**:27, 1929.

11. Kunz, H.: Deutsche Ztschr. f. Chir. **249**:109, 1937.

12. Mallory, F. B.: New England J. Med. **207**:843, 1932.

13. Catron, L.: Arch. Path. **11**:847, 1931.

14. Eggers, C.: Am. J. Surg. **27**:52, 1935.

15. Schneider, J.: Virchows Arch. f. path. Anat. **252**:706, 1924.

16. Lichtenstein: Deutsche Ztschr. f. Chir. **233**:24, 1921.

17. Lyssunkin, L. L.: Frankfurt. Ztschr. f. Path. **46**:107, 1934.

18. Neumann, N.: Arch. f. Kinderh. **98**:139, 1933.

19. Nevinny, H.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **40**:277, 1927.

20. Sala, A. M.: Arch. Path. **9**:950, 1930.

21. Pallasse, E., and Roubier, C.: Ann. de méd. **3**:243, 1916.

and Rabin in one of their cases of circumscribed pleural neoplasms. There was no evidence for a neurogenic origin of the tumor. The right phrenic nerve passed into the tumor but remained well separated from it. At the diaphragmatic side, single small nerves were seen entering the tumor, which showed no relation to the tumor tissue and broke up into fine branches, which gradually disappeared. Furthermore, the definite fascicular arrangement of the connective tissue fibers, the presence of smooth muscle fibers and the absence of palisades and whorls speak against a neurofibroma. For comparison, a neurofibroma

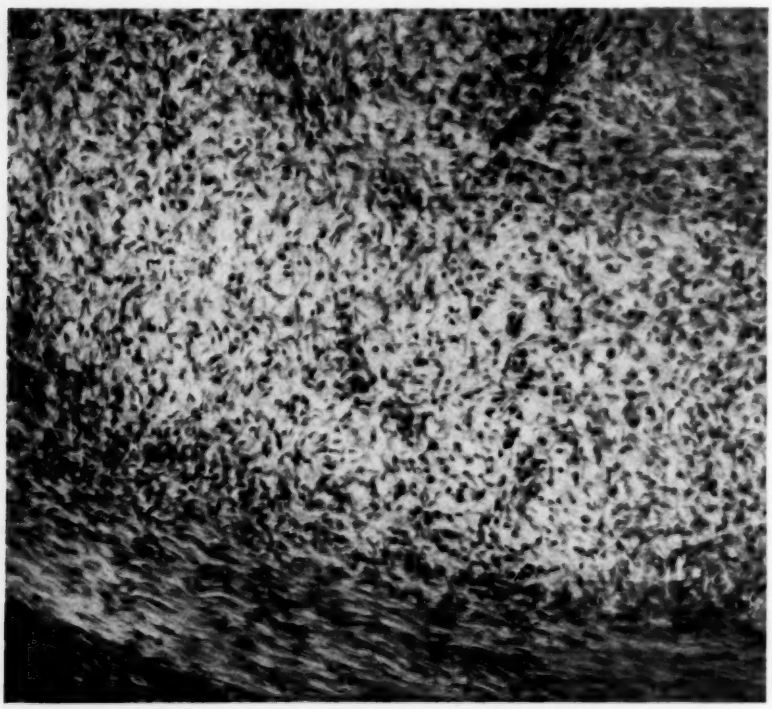


Fig. 6.—Neurofibroma of an intercostal nerve; hemalum, eosin; × 150.

of the third intercostal nerve is pictured (fig. 6). It was found bulging into the right pleural cavity of a colored woman of 51 years who was suffering from generalized neurofibromatosis.

The tumor of the diaphragmatic pleura showed much hyalinization and calcification as well as focal areas of ossification. Calcification of pleural fibromas has been described by Schmidt and Foltz. In the periphery of the tumor, glandlike structures were found which I attribute to mesothelial cells of the pericardium that became included in the tumor. These glandlike structures resemble the proliferations of mesothelial cells often seen underneath pericarditic or pleuritic fibrinous membranes undergoing organization. In the second tumor, similar glands were observed near its free surface.

By compressing the inferior vena cava the diaphragmatic tumor had caused severe circulatory disturbances, and the interference with

the outflow of the blood from the lower half of the body dominated the clinical picture. There was marked sclerosis of the inferior vena cava below the obstruction, with much calcification of the veins of the pelvis and lower extremities, which could be seen on roentgenologic examination and caused much differential diagnostic discussion. The case demonstrates that increase of intravenous pressure without complicating inflammation may cause calcification of veins.

The second pleural tumor contained relatively little connective tissue, and smooth muscle fibers were greatly in excess. In circumscribed areas the ground substance was loosened, and mononuclear cells of histiocytic type accumulated. By taking up doubly refractile lipoid mixtures the histiocytes became transformed into foam cells, which in places were so numerous as to produce grossly visible yellow areas. The tumor resembled closely the "xantho-leiomyoma" described by Wessén.[9] Xanthomatous changes have been observed in granulomatous lesions as well as in different types of benign and malignant tumors. The relation between xanthoma and tumor was recently well discussed by Weidman,[22] who emphasized that xanthoma should be differentiated from the foam cells which appear in tumors secondary to local degenerative or suppurative processes. He was inclined to attribute the formation of xanthomas in tumors to a "coexistant fatty dyscrasia," for the presence of which he failed to give satisfactory proof. Thus in my case there were no signs of disturbed cholesterol metabolism, and the usual sites of xanthomas, such as the skin, tendons, joints and lungs, were not affected. I believe that local xanthomatosis of tumors and granulomas is due to a peculiarity of their metabolism and that local lymph stasis greatly favors its development. In the sections, one can follow the formation of the xanthomatous areas. It starts with local edema, followed by an accumulation of free histiocytes which, in turn, by storing lipoid granules, assume the appearance of xanthoma cells. Between the cells, tiny free sudanophilic granules can be seen in the edematous ground substance.

As far as the source of the pleural leiomyoma is concerned, the smooth muscle fibers of the pleural blood vessel or of the pleura itself (Baltisberger[23]) have to be considered. An origin from the latter appears more likely, and the location of the second tumor suggests an origin from the pleuro-esophageal muscle.

The question will arise as to the relation between the pleural tumors and the neoplastic processes in the lymph nodes. It has already been said that the pleural tumors did not show any local evidences of malignancy. There was no invasion of capsule, of the wall of the chest or of blood vessels. There were no areas of abnormal cellularity nor any nuclear anaplasia. The first tumor revealed no mitosis, and in the second tumor, after long searching, a single mitosis with regular chromosomes was found. The tumors did not differ principally from the benign leiomyoma of Kornitzer,[7] while their microscopic picture was very different from the leiomyosarcoma described by Catron.[13] In view of the apparent benignity of the tumors of the pleura the interpretation of the tumors of the lymph nodes as metastases may meet objection, as the occurrence of benign metastasizing leiomyomas in other

22. Weidman, F. D.: Arch. Surg. **34**:792, 1937.

23. Baltisberger, W.: Ztschr. f. Anat. u. Entwcklngsgesch. **61**:249, 1921.

locations has been questioned. I believe, however, that the weight of evidence is in favor of a metastatic origin.

First, the structure of the tumors of the pleura is identical with that of the tumors of the lymph nodes. One of the pleural tumors showed a tendency toward calcification and ossification and so did the tumors of the lymph nodes. The other pleural tumor showed xanthomatous areas and so did the tumors of the lymph nodes. Second, the blastomatous changes were confined to the lymph nodes which received their lymph from the areas in which the pleural tumors were located. Third, in the first case early stages of the neoplastic transformation of the lymph nodes could be seen which resembled early metastasis; that is, the tumor cells appeared in the sinuses and by increasing in number encroached on the lymphadenoid tissue, gradually replacing it. The calcified nodules in the indurated fat tissue about the right adrenal were probably also metastases.

The arguments which I have brought forward in favor of a metastatic origin of the tumors in the lymph nodes exclude, I believe, the other possibility, that of independent simultaneous growths. I may add that, according to my knowledge, multiple fibromyomas of the lymph nodes have never been described. That the fibromyomas of the pleura and periaortic lymph nodes of my second case may be metastases from the uterine myomas can be excluded. The uterine myomas were rich in fibrous tissue and did not contain xanthomatous areas. If uterine myomas metastasize they select chiefly the lungs, and the lymph nodes are spared.[24] In Deussing's case of multiple myomas of the lung there were also myomas of the uterus, but Deussing did not link the two together and thought of independent tumors.

The two cases which I have reported strongly support the contention that certain tumors which are histologically benign may occasionally produce metastases to distant organs. In this respect the pleural fibromyomas may behave like the myomas of the uterus or stomach (Melnick[25]). With the great progress in thoracic surgery pleural tumors are coming to operation in increasing numbers, and it may be wise to keep in mind that some of these tumors may metastasize although they are histologically benign.

SUMMARY

Two cases are reported of a large tumor of the parietal pleura composed of smooth muscle fibers and connective tissue. In one instance the tumor showed much calcification and focal ossification and caused the patient's death by compressing the inferior vena cava. In the other, the tumor contained xanthomatous areas and was incidentally found at necropsy in a woman who had died from cancer of the uterus. In both instances the tumor was histologically benign. In both, neoplasms identical in structure with the pleural tumor were present in lymph nodes draining lymph from the region in which the pleural tumor was located. The weight of evidence favors a metastatic origin of the tumors of the lymph nodes.

24. In this connection it may be mentioned that according to R. A. Willis (The Spread of Tumours in the Human Body, London, J. & A. Churchill, Ltd., 1934) leismyosarcomas rarely yield lymphatic metastases.

25. Melnick, P.: Am. J. Cancer **16**:890, 1932.

CEREBRAL CHANGES IN AMAUROTIC FAMILY IDIOCY (TAY-SACHS DISEASE)

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Although numerous reports and discussions of amaurotic family idiocy have appeared in the literature, the pathogenesis of this condition remains as yet uncertain. This is readily appreciated from the numerous controversial opinions concerning the relationships of this disease, particularly to essential lipoid histiocytosis (Niemann-Pick disease). Because of the uncertain status of the condition, it seems worth while to add any observations that might help in an understanding of this interesting disease.

REPORT OF CASE

A white girl, first seen at the age of 9 months, was unable at that time to sit up and showed moderate weakness and incoordination of all her limbs. There was also some difficulty with vision.

The history indicated that the labor and birth were entirely normal. She had been healthy when born and had developed in a normal manner to about the age of 7 months. At that time she was not doing well. She stopped gaining weight, refused food and soon lost her ability to grasp objects. It became apparent that retrogression was taking place.

She was carefully observed for eighteen months, until the time of her death. There was progressive weakness of the muscles of all the extremities, the limbs terminally becoming useless. The extremities as a rule were spastic. There was gradual loss of vision, progressing to a state of total blindness. Ophthalmoscopic examination revealed a cherry-red spot in the region of the macula lutea.

The child was admitted to the hospital at the age of 21 months, at which time she was suffering from major and minor convulsive seizures. She was dehydrated and seemed to have some paralysis of the muscles of deglutition. Swallowing food seemed to cause paroxysms of vomiting. It was necessary to feed by tube. An encephalogram failed to show evidence of pathologic lesions.

The blood sugar was 72 mg. per hundred cubic centimeters. Studies of the blood gave entirely normal values. The blood fatty acids amounted to 156.4 mg.; free cholesterol, to 61 mg.; cholesterol esters, to 110 mg.; total cholesterol, to 180 mg. The cerebrospinal fluid pressure was 23 mm. of mercury.

The condition was progressive and death occurred suddenly at the age of 27 months.

A careful check of the family history over three generations failed to reveal any Jewish parentage. The father, 27 years old, was of French descent; the mother, 24 years old, of French and Irish descent. Both parents were healthy. There had been no other pregnancies and no other similar cases in the family of either parent.

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A complete necropsy was performed. The organs were grossly normal with the exception of the central nervous system. The brain and spinal cord were of a peculiar rubbery consistency. It was almost impossible to crush the brain tissue; like rubber, it returned to its original shape on release of pressure.

Sections were removed from various areas of the cerebrum, cerebellum, midbrain, medulla and spinal cord. These were stained with various stains to bring out the structure of the cell bodies and nuclei.

Ganglion Cell Changes.—The ganglion cells throughout the cerebrum revealed extensive changes, which consisted primarily in rounding-up of the body and swelling or ballooning-out of the cell (*A* in the figure). Many cells were only moderately enlarged, the Nissl substance around the periphery of the cell was absent, and the cytoplasm stained faintly. In the more swollen cells, all the Nissl substance had disappeared and the cytoplasm became less and less distinct, the entire cell body eventually appearing colorless. Some cells were greatly swollen. These contained a finely granular material distributed uniformly throughout the cell body. These granules were frequently hard to visualize with most stains but were usually differentiated readily with Weigert's hematoxylin (*A* in the figure). Thus nerve cells of two distinct types were seen: swollen ones without granules and swollen ones filled with fine particles.

The nuclei showed strikingly little change with the exception that they became eccentric in the most altered cells.

The cell processes were usually detached from the cell bodies, and for this reason the large ballooned cells presented a most bizarre picture, appearing pyriform, elliptic, oval or elongated. The processes showed changes similar to those in the cells themselves. They were frequently swollen and were either clear or filled with finely granular material. Some processes could be found attached to the cell body. In many instances these attached dendrites were already swollen and partially filled with tiny particles while the cell body itself showed little or no change. On the other hand, many ganglion cells underwent extensive alteration while their cell processes remained small and apparently normal. It appears, therefore, that these changes may attack any part of the entire cell unit with little regard to the portion of the cell structure first involved.

Most of the dendrites were found detached from their cells, appearing as scattered masses throughout the cortex. They were often globular or elliptic acellular structures the origin of which would have been doubtful had it not been for the occasional dendrite of similar character that was still attached to its cell body.

Actual disappearance of ganglion cells seemed to be uncommon. In the frontal regions the cell processes were only slightly altered; most of the changes appeared to be in the cell bodies. This was in distinct contrast to the changes in the occipital regions, where the most extensive changes were observed in the dendrites. Here cystic swelling of the processes could be found amid slightly to moderately altered cells.

The basal nuclei presented changes quite similar to those in the rest of the brain. The small cells of the putamen and caudate nucleus, as well as the giant cells of the globus pallidus, were moderately enlarged. Their dendrites were not altered. These cells did not contain granules. The cells of the thalamus and of the hypothalamic nuclei were swollen and showed changes similar to those seen in the cortical cells. The alteration was not limited to any particular cell group but involved them all quite uniformly. The extensive dendritic changes in these regions caused the dendrites to appear vacuolated.

Histochemical investigation of the intercellular content was interesting. Scarlet red failed to stain most of the ganglion cells. An occasional ballooned cell contained a few faintly yellow-staining granules. Nile blue sulfate and Ciaccio's stain likewise failed to reveal any particles within the swollen cell cytoplasm. However, after mordanting with Weigert's mordants and staining with hematoxylin, the sections revealed numerous fine black particles filling portions or all of many of the enlarged ganglion cells as well as their swollen dendritic processes (*A* in the figure). From its staining properties it may be assumed that the composition of the intercellular fat was probably something between that of normal myelin and neutral fat and that it may be called a hematoxylinophilic material. Many of the ganglion cells did not contain these granules although they were quite swollen and showed the usual structural changes. Cell processes as a rule contained much less hematoxylinophilic granules than did the cells themselves.

Astrocyte Changes.—Changes occurred within the macroglia cells, but these were by no means as marked as the alterations occurring within the ganglion cells. There was a moderate increase of these cells in the cortical areas, but no true subpial gliosis was found such as Schlesinger and his associates[1] described in their case. There was marked alteration in the structure of the astrocytes throughout the gray and white substance. Their cell bodies were moderately swollen, and their dendrites were usually absent, giving these cells a rounded appearance. As a rule, the ballooning-out of the astrocytes did not reach great proportions, and their nuclei remained intact. Occasional cell processes remained attached to the cells, and these were normal in appearance or at most only slightly swollen. Changes within the macroglia cells were observed in all regions of the cerebral hemispheres and did not seem to be more marked in any particular area. With scarlet red many more astrocytes than ganglion cells were stained. Throughout the gray and white substance could be found many macroglia cells that appeared dull yellow with this stain. Frequently even the attached processes became impregnated. Weigert's stain revealed a distinct scarcity of granules in the astrocytes. Apparently within these cells the degree of alteration had not advanced to the stage of deposition of hematoxylinophilic granules. A few of the largest cells contained peripherally a few granules, but in no case could any of these granules be found within the processes of these cells.

The oligodendroglia cells revealed no changes and appeared unaffected by the changes about them.

Microglia Cell Changes.—The microglia cells participated in the pathologic changes and discharged their functions as active scavengers. The most marked alterations in these cells were seen in the cerebral cortex, where the cells were observed in all stages of metamorphosis from typical microglia into rod cells and finally into the globular scavenger cells. The fat granule cells were scattered between the altered ganglion cells and astrocytes and were engaged in the removal of cerebral débris. They varied in size from small cells to large irregular multinucleated protoplasmic masses. Their cytoplasmic contents were very coarse and stained more deeply than the fine, barely visible granules of the cells of neuroectodermal origin. With scarlet red the content of these cells stained deep orange and was beyond doubt of a different nature from that of the surrounding nerve cells.

A most striking picture was presented by these scavenger cells within the internal capsule, where they had collected in such large number that they replaced much of the tissue. This will be discussed in a later paragraph.

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1. Schlesinger, B.; Greenfield, J. G., and Stern, R. O.: Arch. Dis. Childhood **9**:1, 1934.

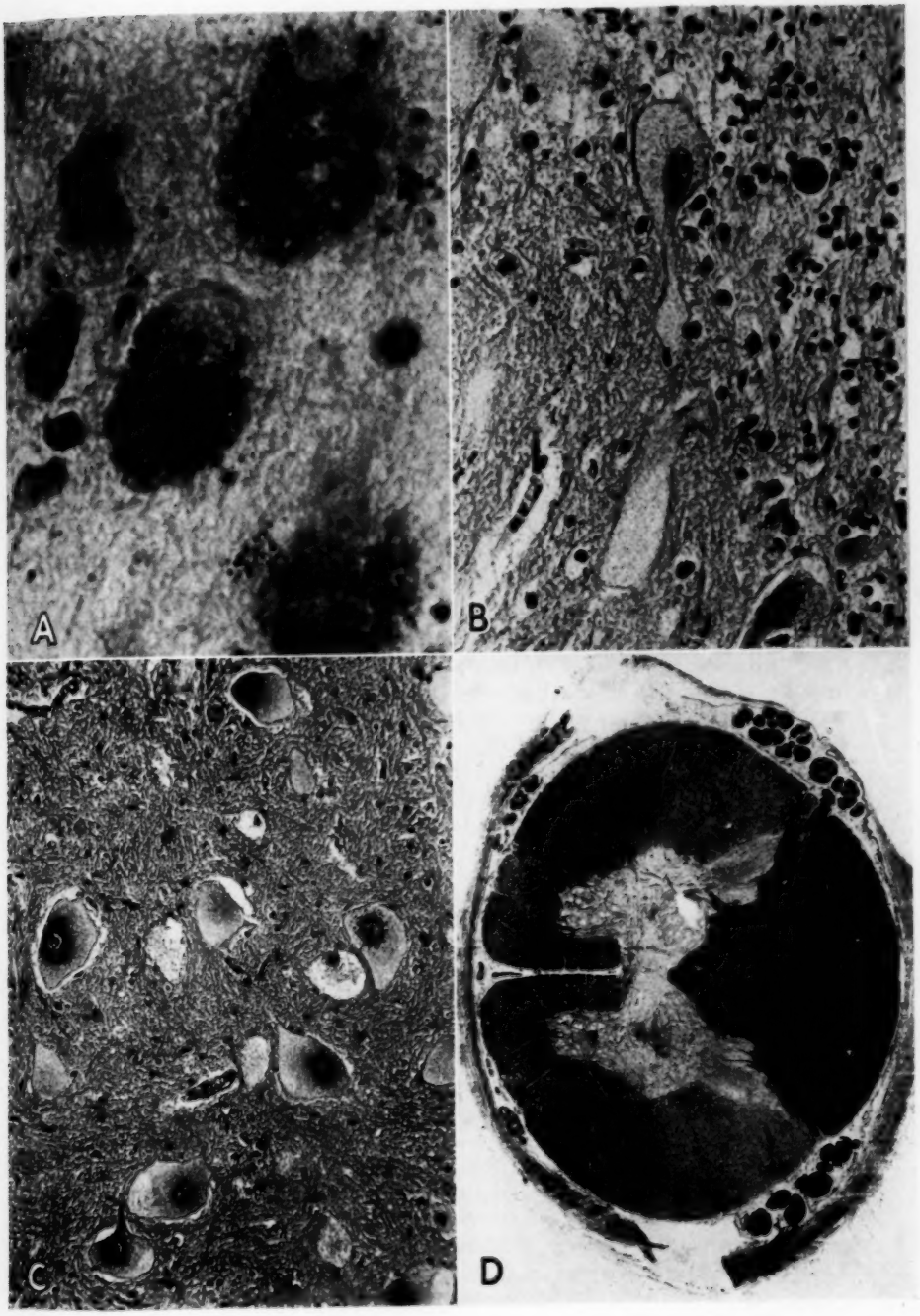
EXPLANATION OF PLATE

A, ganglion cells from the cerebral cortex; Weigert's stain. These cells are swollen, rounded and filled with many dark granules. In each of these cells the nucleus is displaced to the periphery.

B, section through the cerebellum; hematoxylin and eosin stain. Note the enlarged Purkinje cell with a moderately swollen dendrite still attached to the cell body. Other detached processes can be seen in the molecular layer. A few fat granule cells are present in the vacuolated granular layer.

C, enlarged nerve cells in the anterior horn of the spinal cord; hematoxylin and eosin stain. Some cytoplasm still surrounds the nucleus. Most of the cell processes are not well visualized.

D, section through the thoracic cord; Weigert's stain. Note the extensive changes in the lateral columns, the milder involvement of the anterior columns and the apparently intact posterior funiculi.


A
B
C
D

It is readily apparent from the description of these cellular changes that the cerebral cortex in this case of Tay-Sachs disease presented a most striking picture with this scene of ganglion cells, astrocytes and microglia cells in all stages of alteration.

Myelin Sheath Changes.—Even with the hematoxylin and eosin stains one was impressed with the altered appearance of the white substance throughout the brain tissue. The deficiency in myelin staining was most readily apparent after Weigert staining. The entire brain appeared somewhat pale although the general topography was well preserved. In many areas myelin was entirely absent. Some of the demyelinated tissue contained numerous fat granule cells in various stages of activity. However, equally numerous regions were found showing no scavengers in spite of the complete absence of myelin sheaths. In fact no signs of the previous presence of such cellular activity could be made out in these regions.

The internal capsule had practically disappeared and appeared totally white with the Pal-Weigert stain. This region was, however, filled with scavenger cells. Phagocytes of all shapes and sizes could be observed filled with coarse granules, the latter probably the end-product of cerebral degeneration. With the scarlet red stain these cells were found to be full of particles staining deep orange. All the fat was intracellular, and the extreme engorgement of these phagocytes made a detailed study of their structure difficult. The contents of these cells did not stain with Weigert's hematoxylin. It was apparent from these observations that the internal capsule had been fully developed and was now undergoing extensive demyelinization.

Cerebellum.—The Purkinje cells of the cerebellum showed alterations similar to those already described for the ganglion cells elsewhere. Their flask-shaped bodies were moderately swollen, becoming oval, elliptic and elongated (*B* in the figure). The swelling of these cells was, however, not as marked as that of the nerve cells elsewhere in the nervous system. The nuclei remained unchanged. Dendrites could frequently be followed from the cell body to the molecular layer of the cerebellum (*B* in the figure). These processes were only moderately swollen. Occasionally a greatly distended dendrite could be found. The entire molecular layer was filled with isolated faintly staining areas that had a structure similar to the distended processes of the Purkinje cells. These structures no doubt represented detached dendrites that had become so markedly altered that they could no longer be identified. Almost all the altered Purkinje cells and their processes were filled with hematoxylinophilic granules.

The granular layer of the cerebellum revealed only minor changes. The granular cells were unaltered. The white lamina showed extensive vacuolation and myelin sheath destruction. The inner portion of the granular layer lying adjacent to the demyelinated white substance was filled with actively phagocytic fat granule cells that apparently were attempting to remove the degenerating myelin tissue.

Spinal Cord.—The dura was thickened but showed no signs of cellular activity. There was enlargement of all the nerve cells, particularly of those of the anterior horns (*C* in the figure). These had become more swollen than any cell observed anywhere in the nervous system. Every cell of the cord showed alterations of some degree. In the moderately swollen cells, the bodies were rounded and the cytoplasm peripherally faded while the centrally placed nuclei remained intact and were surrounded by large granules of heavy cytoplasm (*C* in the figure). As the cells continued to swell, the cytoplasm around the nuclei became fainter and finally lost its tinctorial properties. These large ballooned-out cells of the spinal cord appeared to be filled with some type of material, the nature

of which we were unable to determine. The processes of these cells were not greatly altered. Those attached to the cell bodies tapered off a short distance from the cells.

Weigert stains showed extensive changes within the white substance of the cord. The pyramidal tracts and the dorsal spinocerebellar tracts were completely demyelinated, while the remainder of the lateral columns, as well as the anterior columns, showed a partial but quite uniform decrease of myelin (*D* in the figure). The changes in the pyramidal tracts were correlated, of course, with the extensive changes in the Betz cells as well as with those in the internal capsule. Only the posterior columns failed to show changes with the Weigert stain (*D* in the figure). No cellular activity was observed within the demyelinized areas. The rootlets were intact.

The blood vessels and the leptomeninges of the nervous system were entirely normal. Careful search failed to reveal any of the foam cells so characteristic of Niemann-Pick disease.

COMMENT

To recapitulate briefly, in our case of Tay-Sachs disease there was exhibited swelling of almost all the ganglion cells associated with a balloon-like swelling of their dendrites. The extent of the pathologic process could not be appreciated, however, until Weigert's myelin sheath stain was applied. This revealed involvement of almost every nerve cell. No doubt this cell alteration accounted for the complete demyelinization of various areas of the nervous system.

Two distinct changes occurred within the nerve cells. The first consisted of swelling of the cells and fading-out of their cytoplasmic material. This process seemed to be followed by the deposition within the cell body of fine hematoxylinophilic granules. The condition of the large nerve cells of the spinal cord apparently never advanced beyond the stage of swelling. A deposit of granules was not found in these. Dendritic changes occurred before, simultaneously with or after alterations within the cell body. Rarely did the dendrites acquire granules within the cerebellum. The astrocytic involvement was similar although less marked than that of the ganglion cells. It is interesting to note the striking selectivity of the pathologic process in this disease, which was confined to the neuro-ectodermal elements of the central nervous system.

The pathologic changes in this disease may serve as the basis of an opinion as to the relationship of this condition to Niemann-Pick disease. The latter is a primary metabolic disturbance which leads to an overloading of the blood and tissues with lipoid material, chiefly phosphatides. Many investigators, such as Pick,[2] Spielmeyer,[3] Bielschowsky[4] and Kufs,[5] have expressed the belief that amaurotic idiocy has as its basis the same metabolic disturbance. However, in Tay-Sachs disease there is localization of the general lipoid disturbance to the central nervous system alone. These authors believe that the first alteration in the nervous system is the deposition of fat within the cells, with secondary swelling of the cells. Apparently such a process did not occur

2. Pick, L.: Am. J. M. Sc. **185**:601, 1933.
3. Spielmeyer, W.: J. f. Psychol. u. Neurol. **38**:120, 1929.
4. Bielschowsky, M.: J. f. Psychol. u. Neurol. **26**:123, 1920.
5. Kufs, H.: Ztschr. f. d. ges. Neurol. u. Psychiat. **145**:565, 1933.

in our case. The deposition of lipoid was not the first change but was invariably preceded by marked swelling of the cells. From a histologic point of view we are inclined to agree with Schaffer,[6] who maintained that Tay-Sachs disease is an endogenous condition involving only the ectodermal elements of the brain. There results a primary degenerative condition with swelling of the nerve cells, which later become filled with granular prelipoid material.

The white substance is as a rule little affected in Tay-Sachs disease. Alteration of myelin sheaths is rare, although it has been described by Bielschowsky,[4] Ostertag,[7] Globus,[8] Bertrand and van Bogaert[9] and Brouwer.[10] Bielschowsky and Brouwer were under the impression that the absence of myelin represented poor development of the sheaths and not demyelinization. Bertrand, on the other hand, believed that demyelinization occurred.

It appears that both processes may occur. There can be no doubt that destruction of myelin occurred in many areas of the brain of our patient. It was most marked in the internal capsule and in the cerebellum. Here the presence of areas of myelin with demyelinized débris, as well as of extensive proliferation and activity of the fat granule cells filled with phagocytosed cerebral débris, warrants no other conclusion. On the other hand, numerous completely nonmyelinized areas were present in which no signs of myelin sheath destruction could be detected. It seems hardly possible that these regions had become demyelinized and the débris completely removed without leaving any signs to indicate the occurrence of such a process. A more logical conclusion is that myelinization of these areas had never occurred.

SUMMARY

A case of Tay-Sachs disease in which there was extensive involvement of the central nervous system is reported. The most striking changes were found within the tissues of neuro-ectodermal origin. These changes consisted of primary swelling of the cells and their processes, followed by deposition of hematoxylinophilic granules within many of the more extensively altered cells. Many areas throughout the white substance of the brain showed absence of myelin. In some of these regions demyelinization had occurred, while in others it appeared as if myelinization had never taken place.

The spinal cord revealed almost complete destruction of the lateral columns and partial involvement of the anterior columns. The posterior funiculi were intact.

As a result of these pathologic studies, we are inclined to agree with Schaffer, who believes that Tay-Sachs disease is an endogenous condition involving the ectodermal elements of the brain and is not related to Niemann-Pick disease.

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6. Schaffer, K.: Deutsche Ztschr. f. Nervenh. **135**:11, 1935.
7. Ostertag, B.: Zentralbl. f. d. ges. Neurol. u. Psychiat. **39**:190, 1925.
8. Globus, J. H.: Ztschr. f. d. ges. Neurol. u. Psychiat. **85**:424, 1923.
9. Bertrand, I., and van Bogaert, L.: Encéphale **29**:505, 1934.
10. Brouwer, B.: Proc. Roy. Soc. Med. **29**:579, 1936.

Laboratory Methods and Technical Notes

GUM DAMMAR FOR MOUNTING SECTIONS

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My co-workers and I have been using gum dammar as a routine in the mounting of permanent microscopic tissue sections for a period of about nine years. In this period we have prepared approximately 200,000 sections. This mounting medium has proved to be superior in every way to Canada balsam, which was formerly used. It is my impression that in the majority of laboratories in this country balsam is still used. I believe an effort should be made to inform pathologists of the advantages of gum dammar.

The possibilities of this material were first called to my attention by Dr. Francis Carter Wood, who recommended its use for the routine preparation of histologic specimens, saying he had used it exclusively for a number of years and found it entirely satisfactory.

I have recently examined a number of older slides, including some mounted ten years ago in Canada balsam and others prepared in the immediately succeeding months with gum dammar. The slides mounted with gum dammar have the advantage in every way:

1. The balsam slides are much dirtier and mussier in appearance.
2. They dry slowly and after years of curing still have a tendency to stick together when stored in contact.
3. The balsam has turned yellow at the edges and under the cover slips.
4. The edges of the sections within a zone 2 or 3 mm. wide at the edge of the cover slip are completely decolorized as far as the nuclear stain is concerned.

On the other hand, the slides mounted with gum dammar are much cleaner, dry quicker, will not stick together if allowed to dry for a few months before storing in contact with other slides, do not discolor and do not fade.

An important advantage is the possibility of storing slides in compact arrangement, since they have no tendency to stick to one another, thus saving much space and expense in providing cabinets for the permanent filing of the slides.

Prepared solutions of gum dammar ready for use can be purchased from laboratory supply houses. Since we began its use, it has been prepared in our own laboratory. Its satisfactory preparation is simple. The gum dammar is purchased in a crude state from drug houses and is dissolved in xylene.

PREPARATION

Dissolve the resin in the "neutral histological (practical) xylene" specially prepared by the Eastman Kodak Company. This is done by placing the ingredients

From the Pathological Laboratory of the Los Angeles County Hospital.

together in a large stoppered bottle in an incubator and stirring with a glass rod several times daily until the mixture is of a heavy syrupy consistency. This is usually accomplished in about a week's time. Then strain through four layers of clean gauze and store in a dark bottle. If the resultant solution is found to be too thin for mounting purposes, a thicker consistency can be secured by placing the unstoppered bottle in an incubator for evaporation until it is of the right consistency. A satisfactory solution is considerably thinner and much less sticky than the preparations of Canada balsam generally used, and can be applied to the slide quickly.

General Review

RECENT CONTRIBUTIONS TO THE IMMUNOLOGY OF HELMINTHIC INFECTIONS

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CONTENTS

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There is at present essential agreement among investigators that immune phenomena are elicited in animals as a result of infection with the helminths, just as with other groups of infectious agents. Certain species of animals are absolutely resistant to infection with a given helminth, just as hosts are known which cannot be infected with a given bacterium or filtrable virus. Furthermore, those animals which are infected by helminths bring humoral and cellular forces to bear against the parasites similar to those that come into play with other types of infections, these agencies being probably responsible, as likely they are

with the other kinds of infections, for the resistance shown to superinfection. Often the existence of a helminthic infection can be proved by demonstrating antibodies in the blood serum or by carrying out a skin test along conventional lines, the results obtained by these methods being characterized by their usual relative specificity. Moreover, the serum from animals with a helminthic infection has in certain cases been shown to manifest protective properties after injection into a normal susceptible host, as is true of the serum of animals with certain bacterial infections. Although it is still too early to disregard the possibility that a fundamental difference in character exists between the immunity developed against helminths and that against other forms, the essentially gross similarities which have just been pointed out and which will be amplified in the succeeding pages suggest, so fas as they go, the identity of the governing principles.

In recent years, many workers have been drawn into the field of immunity to the helminths, and an extensive literature has appeared from their work. With the exception of papers in Russian (Schulz and Shikhobalova) and Italian (di Aichelburg), no comprehensive review of work on immunity to helminths has appeared to which one can turn for a summary of the results since 1929, when Taliaferro offered his invaluable monograph on the subject. It is to supply the need for a survey of the work since 1929 that the present paper has been undertaken.[1]

I. EXPERIMENTAL EVIDENCE ON IMMUNITY

NATURAL IMMUNITY

Natural immunity against a parasitic helminth may be lifelong or may be acquired only as the host grows older. Often the insusceptibility or resistance to the parasite is confined to a breed within the host species or even to a strain within the breed. It appears that the genetic constitution of the host, and perhaps likewise that of the parasite, governs the incidence, severity and duration of the infection, although other responses of the host directed specifically against the invading parasite also come into play. These different aspects of natural immunity will be dealt with briefly in the following paragraphs.

Comparative Resistance of Different Species and Different Breeds of Animals.—Certain parasites have the capacity to invade many different species of hosts, often of distant relationship, whereas others are strictly limited to one or a few closely related hosts. For example, till

1. Peters has presented in English a survey of recent developments in helminthology related especially to the cestodes, in which some of the immunologic literature is summarized.

the present, Taenia saginata in the adult stage has been recorded found in man alone. Trichinella spiralis, on the other hand, has been found to infect at least twenty-five different kinds of mammals, as well as birds (Matoff, 1936) and possibly salamanders, although it is appreciated that the larvae of the parasite do not reach their full maturity in all of the hosts which they infect. Turkeys are distinctly more resistant to Ascaridia lineata than are chickens of the same age and they are less important than chickens for the dissemination of the eggs of this parasite (Ackert and Eisenbrandt). Similarly, cats are naturally much less susceptible to Ancylostoma canium than dogs, even young kittens being less susceptible to this parasite than old dogs (Scott).

Animals are, in general, intolerant of the parasites of physiologically unrelated animals (Cameron). Fuhrmann emphasized this point to the extent of considering the natural occurrence of a parasite in a new host sufficient to warrant description of a new species, but Meggitt insisted that specificity for host is seldom so limited and listed a considerable number of parasites, including some recorded frequently from man, which occur in hosts of remotely related taxonomic orders. The specificity for host often appears to be relative and to depend on such factors as the food, age, size and condition of the host, the dosage, virulence and sensory faculties of the parasite and the immunity mechanisms and heredity of both the host and the parasite (Becker).

The natural immunity or susceptibility of animals to infection with Echinococcus granulosus seems to depend, in part at least, on the action of digestive juices, particularly those of the intestine. Berberian found that fresh viable evaginated hydatid scolices placed in the intestinal juices of sheep, cattle and man at 37 C. were completely digested, but that when they were placed in the juice of the dog or the cat at this temperature they were not digested. This finding appears to explain why the parasite in its adult stage is able to infect only the dog and the cat among those species of hosts included in this study.

The converse of Berberian's experiment—namely, the demonstration of a digestive substance in the parasite's body which is active on the tissues of the specific host—has also been demonstrated recently. Davis (1936a) found such a substance in an extract of cercarial bodies, which he demonstrated in an in vitro experiment. Sedimented cercarias of Diplostomum flexicaudum were triturated with distilled water in a mortar, kept over night in a refrigerator and filtered the next morning. Small pieces of tadpole muscle, skin, and tip of the tail, which cercarias of this type are known to invade, were covered with the extract in a Stender dish and placed in the icebox for three hours. Marked changes were at once apparent on examination: The affected parts were swollen, frayed, loosened and covered with a filmy layer; the muscle fibers were thinner and separated from each other, and the nuclei were faded, enlarged and disintegrated. The specificity of the reaction has not yet been studied, although it is appreciated that certain changes which occur in the skin of frogs and toads at metamorphosis render

them subsequently immune to penetration by the cercarias (Davis, 1936b). The substance responsible for the action is relatively thermostable, being unchanged after exposure for one hour to temperatures from 60 to 80 C.

A marked decrease in specificity for host has been effected by Nakajima by treating larval Ancylostoma duodenale with an emulsion of the tissue of the normal host. Larvae from the lung of a puppy were treated with an emulsion of human lung tissue, then administered to rabbits. Whereas normal larvae not so treated failed to develop in rabbits, those treated with human lung developed to the point of sexual differentiation just before the fourth ecdysis. Larvae treated with an emulsion of rabbit or guinea pig lung showed no such development. These results indicate that biologic significance must be attached to passage of the larvae of the ancylostome through the lung of the specific host.

Evidence has also been adduced for a difference in susceptibility to parasites in certain breeds within a given host species. Heavy breeds of chickens (Rhode Island Reds, Plymouth Rocks) seem more resistant to Ascaridia lineata at all ages than lighter breeds (Leghorns, Buff Orpingtons, Minorcas). Among Minorcas, heavier strains proved more resistant to this parasite than lighter ones (Ackert, Eisenbrandt, Wilmoth, Glading and Pratt).

A mixed flock of sheep kept overcrowded in a pasture (from 30 to 40 per acre) and infected with a variety of helminths were studied by Cameron for evidence of natural immunity to helminths. Cheviots were more tolerant, as judged clinically, of the effects of the parasitism and were less heavily infected than lowland breeds, such as Border Leicesters, with Shetlands and Scottish blackface sheep intermediate.

Acquisition of Immunity with Age.—The animals of some host species become naturally resistant to a given parasite only as they grow older, younger animals either lacking the defense mechanisms which the older ones possess or else providing a more suitable milieu in which the parasite can develop. In these species, the parasites which the younger susceptible host has acquired are usually expelled when the host reaches the resistant age. Sandground set forth the postulate that age resistance is shown by a host typically against only abnormal, newly acquired or imperfectly adjusted species of parasites.

(*a*) Trematoda. Although little experimental work on age resistance against trematodes has been reported, it has been noted that schistosomiasis is not often found in persons over 40 years of age (Dixon). This may be in part explained by the natural resistance which develops as the person matures (Fisher) without relationship to infection with the parasite. However, it appears likely that in most cases the age factor and the specific response to the infection are supplementary in function.

The black angelfish, Pomacanthus arcuatus, according to Nigrelli and Breder, shows an inverse age immunity with respect to the ectoparasitic monogenetic trematode Epibdella melleni, this host being susceptible to infection only after the first two weeks of life. Cable noted in herring gulls some evidence of age resistance against the cloacal trematode Parorchis acanthus.

(*b*) Cestoda. Mice and rats manifest age resistance against Hymenolepis fraterna. Both species are very resistant to the parasite during the period of nursing, as well as after from 5 to 7 months of age (Shorb; Hunninen). Recently Ackert and Reid demonstrated in white leghorn chickens age resistance against the cestode Raillietina cesticillus. Fifteen chicks from 20 to 51 days old which were fed 50 cysticercoids showed 4.87 tapeworms per bird, whereas 27 fowls from 71 to 150 days old had an average of 3.22 worms per bird after the same dose.

(*c*) Nematoda. Although no resistance is acquired with age by man against the hookworms that infest man, older dogs are more resistant to the dog hookworm Ancylostoma canium than younger animals (Herrick; Scott). In adult dogs the worms require a longer time to attain sexual maturity, and a smaller percentage of the larvae which penetrate become mature. Sarles (1929b and c) found that oral and cutaneous administration of 20,000 larvae of Ancylostoma to old dogs yielded only 25 adult worms, whereas this dose given to younger dogs produced over 1,000 adult worms. Sarles (1929a) also showed age resistance against Ancylostoma braziliense in cats, manifested by the greater time required for worms to reach maturity in adult cats as compared with that in kittens, by the smaller egg counts in adult cats and by the smaller percentage of larvae which develop in adult cats (3.96 per cent) as compared with the percentage in kittens (32.34 per cent) following a standard dose of infective larvae. Furthermore, the natural life of the worms in the adult cats was less than two weeks, compared with periods up to thirty-two days in young forms. Sarles (1929b) noted also that the local reaction in old dogs to the penetration of hookworm larvae is severe, with prolonged inflammation, in contrast to the slight transient effect in young animals. The larvae were retained in the skin much longer in old dogs than in young ones, and in sections of the skin of older hosts they often showed evidence of partial disintegration. Foster (1935), however, recently questioned the increase of natural resistance to Ancylostoma canium as dogs become older. He considered that a complex of factors which governs the general health and well-being of the animal governs the resistance to invasion by the hookworm. More recently he (1936b) pointed out an inverse correlation between the resistance of dogs and cats and anemia occasioned by hemorrhage or by feeding a milk diet, which is deficient in iron. He suggested that age resistance is related to the natural age curve of the hemoglobin level of a given host. Foster and Cort (1935) noted, as will be discussed later, that young animals were more susceptible than older animals to the effect of a deficient diet in decreasing resistance to Ancylostoma canium, as shown

by an increase in the rate of development and in egg production. Young puppies, moreover, do not have the resistance of older dogs to the human hookworm, Ancylostoma duodenale.

While the infection with no other nematode has been studied so extensively with respect to immunity acquired with age as the hookworm infestation of dogs, a number of other forms have been used in experiments. Rats show age immunity to Nippostrongylus muris (Africa; Chandler, 1932a). Chandler (1932a) obtained his evidence from counts of adult worms made at autopsy following percutaneous infection with a standard dose of larvae. About one-third less worms were recovered from older rats (from 5 to 12 months old) than from younger rats (from 4 to 10 weeks old). Porter (1935a), who also studied age resistance against Nippostrongylus muris, noted that in mice, which are somewhat abnormal hosts for this parasite, there is a very early development of resistance and that there is a longer prepatent period, the development of a smaller percentage of the larvae, a lower output of eggs and a shorter period of infection in mice than in rats.

Foster (1936d and 1937) found that 59 Panamanian equines from 9 to 15 years old had an average of 1,219 strongylid worms per animal, whereas 27 animals from 15 to 30 years old yielded 502 worms per animal. The relationship held for all the commoner species except Habronema spp. and Strongylus vulgaris, the latter of which was equally frequent in all age groups. Hinman and Baker reported in 1,315 New Orleans dogs some evidence of age resistance to Toxocara canis, somewhat less to Trichuris and none at all to Ancylostoma canium, Dirofilaria immitis, Dipylidium caninum and Taenia serrata.

Ross suggested that age resistance is one of a complex set of factors which influence the degree of resistance of lambs to Haemonchus contortus. Kauzal reported that lambs over 5½ to 7 months old show enhanced resistance to the lungworm Dictyocaulus filaria, compared with those less than 2 months old, the latter frequently succumbing to infection.

The possibility of the development of age immunity against Trichinella spiralis was investigated by Matoff (1936), who fed pigeons of different ages with trichinous meat. He found that young pigeons were susceptible to the infection, larval forms appearing in the muscles of the birds, whereas older pigeons showed no invasion of muscles, although the worms which were fed were able to develop to adults in the intestines of the pigeons. He demonstrated (1937) age resistance against Trichinella spiralis in dogs, a smaller percentage of ingested larvae developing to adults in the intestine in old dogs than in young dogs.

The general statement in the literature that only very young chicks are susceptible to infection with Syngamus trachealis has been confirmed by Clapham (1934d), who failed to infect chicks of the age of 10 weeks with this parasite when the birds were on an adequate diet. Waite, however, said that he established the parasite in yearling hens by feeding 150 earthworms harboring Syngamus trachealis, and Morgan (1931a) reported the recovery of 12 pairs of worms from the trachea of a yearling hen.

Clapham (1934b) found that little immunity developed with age in chickens against Heterakis gallinae, although Winfield (1933) showed that in rats resistance developed at a constantly increasing rate with age against Heterakis spumosa, manifested by (1) reduction of the mean worm burden, (2) the percentage of larvae developing from a standard infecting dose, (3) the size of the worms harbored, (4) the percentage of rats infected by a standard dose of eggs and (5) the egg production.

Chickens were found by Ackert, Porter and Beach to exhibit with age increasing resistance to infection with Ascaridia lineata, the resistance reaching a maximum at ninety-three days. In the older groups of chickens, after three weeks of infection worms were shorter than in the younger groups: worms in the forty-five day group of chickens reached an average of 21.19 mm.; those in the sixty-three day group, 16.1 mm.; those in the seventy-one day group, 11.96 mm., and those in the ninety-three day group, 3.93 mm. Morgan (1931b) reported that pigs were susceptible to infestation with Ascaris lumbricoides at the age of 5 months, in contrast to an earlier observation of Ransom that they acquired natural resistance after the fourth month of life.

Genetic Consideration in the Explanation of Natural Immunity.—Very little work has been done on the importance of the genetic constitution of hosts in their resistance to parasitic invasion. Ackert and Wilmoth reported a marked difference in resistance to Ascaridia lineata between a first and a second year flock of Minorca chickens, which they believed due to the introduction of new cockerels for the second year flock. Foster (1935) considered the genetic constitution of dogs significant in the development of immunity to Ancylostoma canium, although other factors affecting the well being of the host likewise affect its resistance to hookworms. Ross and Gordon expressed the belief that certain types of resistance which are shown by sheep against Haemonchus contortus and which are not influenced by nutritional factors are of genetic origin.

It is unfortunate that greater attention has not been paid to the genetic constitution of animals used in experiments in the past and that extended studies on its significance are till now lacking. In most cases, consideration is given to the genetic make-up of the animal only when results are obtained which are not explained by other phenomena, and then the matter is dismissed casually, usually without an attempt to prove that a genetic mechanism intervenes.

Explanation of Natural Immunity by a Specific Influence on Parasites.—In some cases, the resistance manifested by older hosts has been explained as a direct and probably specific effect on the parasite by some factor elaborated by the host. The resistance of older chickens to Ascaridia lineata, for example, may result from the presence of some substance in these birds which inhibits the growth or development of the nematodes (Ackert, Porter and Beach). Such a mechanism has also been suggested as responsible for the resistance of rats to superinfection with Nippostrongylus muris (Chandler, 1936g). The natural destructive property of the fresh serum of certain vertebrates on cercarias may be responsible for the resistance or susceptibility of a given vertebrate to infection with a specific trematode (Culbertson and Talbot; Culbertson). The mucus of certain fish has also been shown to have a similar action on monogenetic trematodes (Nigrelli).

ACQUIRED IMMUNITY

Many species of animals do not manifest natural species immunity against a given parasite and do not acquire resistance naturally as they mature. In such species, however, it is often possible to demonstrate immunity acquired by infection or by artificial immunization with antigens obtained from the specific parasite.

Immunity by Infection.—The most pronounced immune response follows infection with parasites which actually invade the tissues of the host, perhaps to reside for some part of their cycle within the blood stream itself. Forms which are resident only in the intestinal lumen often neither elicit the production of a specific antibody in the serum nor render the host insusceptible to superinfection. I have, therefore, taken full cognizance of the place of residence of the parasite in the body of the host and will subdivide the discussion which follows into parts according to this consideration.

1. Ectoparasites. The monogenetic trematode Epibdella melleni, which is parasitic on the eyes and skin of marine fishes, is able to induce marked immunity in certain of these hosts. Susceptible fish when first introduced into tanks where the parasite is present are subject to heavy infection, which may end fatally. However, after being present in the tanks for several weeks, a considerable number of the hosts show an infection of much less degree, although continually exposed to reinfection (Jahn and Kuhn). Different species, even within the same family of fishes, show marked difference in capacity to manifest the immunity response. Nigrelli and Breder studied the susceptibility and immunity of 57 species of fish to this parasite and have grouped the hosts into four categories with respect to their response: (1) those which acquire partial immunity, (2) those which acquire resistance which persists for a year or more, (3) those infected only during periods of epidemics and (4) those which are always susceptible and show either light or heavy infection. These same authors noted that after recovery from infection at one site certain hosts became reinfected only on new areas of the skin, suggesting that the immunity from the prior infection was relatively localized. Nigrelli showed that periodic injection of powdered parasites into one very susceptible species of fish, Trachinotus carolinus, was of no significance in the building up of immunity, nor was the transfer of serum from immune hosts efficacious in enhancing resistance.

Nigrelli also showed that mucus from naturally resistant fish, e. g., elasmobranchs, kills parasites in vitro in from five to eight hours, whereas mucus from highly susceptible fish, e. g., the pompano, Trachinotus carolinus, permits the survival of parasites for three days—for as long, in fact, as the parasites are able to survive in the control sea water.

2. Parasites in the Intestinal Lumen. Convincing evidence for immunity against organisms which develop and remain within the intestinal lumen has been obtained only rarely. Such organisms, especially if present in but small numbers, seem unable specifically to affect the host sufficiently to call forth a response against the invader. In those cases, however, in which the parasite enjoys a circuit through the blood,

or even merely penetrates the intestinal wall, a more obvious immune effect characteristically follows. In the present subdivision, only those forms will be considered which reside in the intestinal lumen and do not invade either the blood or the fixed tissues in any part of their development.

(*a*) Trematoda. McCoy (1930) reported that a heavy prior infection of snappers with either Hamacreadium mutabile or Hamacreadium gulella does not confer appreciable resistance to a second infection. However, he noted that the parasites were lost in four weeks if the fish were heavily infected, whereas, if but lightly infected, the fish retained the worms for much longer periods, and he considered this an evidence of an immune response by the fish to the presence of large numbers of the parasites. Cable found that an initial infection of the herring gull with Parorchis acanthus did not protect the host against subsequent infections.

(*b*) Cestoda. One of the most satisfactory experiments designed to determine whether the presence of the adult cestode in the intestine confers immunity to subsequent infection was that of Miller (1932g) working with Taenia crassicollis in cats. From the study of 17 experimental and 19 control cats fed cysticerci of the parasite which were recovered from infected rat livers, he concluded that neither the number nor the distribution of the adult worms in the intestine was influenced by prior infection, thus suggesting that neither general nor local immunity resulted from such an infection.

Some other workers, however, who also used parasites which are not invaders of tissue but dwell only in the intestinal lumen, have not reached the same conclusion. Palais concluded that the presence of Hymenolepis diminuta in the rat intestine protected the animal from superimposed infection, as few as 4 worms sufficing to produce this resistance. Yet this worker employed only 3 experimental and 2 control rats, the former having been fed cysticercoids from Tenebrio molitor thirty-two days before they and the controls were given the test feeding. All of the animals were killed and examined six days after the test infection. A considerable degree of immunity has been reported in dogs infected with Taenia serrata against artificial superimposed infection with Diphyllobothrium latum. The latter form is said to develop to the stage of producing eggs but rapidly to die out thereafter (Wigand).

(*c*) Nematoda. Most of the nematodes which have been used in experimental work enjoy a circuit through the tissues and blood before coming to lie as adults in the intestine of their host. Yet, two papers have appeared on immune responses to nematodes which, according to the authors, who also determined the life cycle of the parasites, are not tissue invaders. Clapham (1934b) concluded that no immunity to subsequent infection is conferred on fowls by an earlier infection with Heterakis gallinae. On the other hand, Winfield (1933), from the study of the very closely related form, Heterakis spumosa, in rats, noted fairly definite resistance, manifested by the reduced size and number of the adult worms and by diminished egg production, which was acquired by the twenty-fifth day of an earlier infection. This resistance developed as well with a persistent small infection as with a heavy infection cured through the administration of an anthelminthic (carbon tetrachloride).

Winfield considered that the resistance thus acquired was a specific response to exposure to this parasite and was independent of age immunity, which the rat also showed.

The discrepancy in the conclusions of the two authors is difficult to explain, especially since Baker has pointed out that the form with which Clapham (1934b) worked is during the first four days of its presence in the intestine very closely associated with the cecal mucosa, possibly causing injury to the glandular epithelium. It is possible, however, that some difference exists in the response of the two species of hosts, fowl and rat, the rat being in some way better able to effect an immune response against this type of parasite.

3. Parasites in Tissues and Blood. It is chiefly against parasites which invade the tissues or blood that the body can be expected to respond best immunologically, since these forms affect the host most acutely. The numerous experiments which have been reported on these somatic parasites for the most part bear out this expectation.

(*a*) Trematoda. Ozawa reported the results of experiments in 5 dogs which were infected with Schistosomum japonicum and then, after an indefinite period, cured by antimony sodium tartrate. These animals, together with 5 control dogs, were again, after an unstated interval, exposed to 200 cercarias. At autopsy, about two months later, it was found that on the average, 19.6 per cent of the cercarias to which the previously treated dogs were exposed had developed, whereas 44.8 per cent of the cercarias to which the normal dogs were exposed had developed. The worms in the immune animals were smaller than those in the controls and were degenerated histologically, appearing as though without internal structure.

Several studies have been reported on human immunity to infections with schistosomes, particularly in the Belgian Congo. Fisher expressed the belief that active specific immunity accounts largely for the fact that in heavily infected centers the infection is confined to those less than 30 years of age, with its highest incidence in children of from 5 to 9 years. In communities which are only occasionally exposed to infection, the incidence does not change with age (Fisher). This fact points against the probability that age resistance is a governing factor in the older person's resistance to schistosomes. The absence of the infection in children under 5 years of age is explained by the native custom of washing children in warmed water instead of permitting them to bathe in open streams or pools (Dixon).

Fisher tested the resistance of 6 presumably immune native volunteers in the Belgian Congo by immersing an arm of each in a bath containing living cercarias. After eight and a half months, a few schistosome ova were detected in the feces of 3, the other volunteers remaining constantly negative. Mice that had been exposed to cercarias from the same snails acquired adult male and female schistosomes after the usual length of time required for schistosome growth. Nevertheless, some investigators hesitate to agree that in man immunity to superinfection is acquired by infection with schistosomes (van den Bérghe).

(*b*) Cestoda. Among studies on immunity against superinfection by larval cestodes, the work of Miller and his collaborators is of paramount importance. In 1930, Miller (1930) observed that the presence of one or more relatively large cysts of Cysticercus fasciolaris in the liver of a rat prevented the subsequent infection of the animal with oncospheres of this parasite. Later, he (1931b) determined by experiment that if rats were fed viable oncospheres on two occasions with an interval of five or six months between, the eggs of the second feeding would be prevented from developing. Miller and Massie showed that the immunity which rats acquired as the result of infection with cysticerci persisted for at least sixty days after the surgical removal of these cysts and inhibited almost completely the development of oncospheres fed during this interval. For example, a total of only 3 dead larvae were found following a test feeding of oncospheres in 29 rats from which the cysts of an earlier infection had been surgically removed, whereas in 12 control rats fed the same number of oncospheres an average of 77 cysts per rat developed.

Essentially similar results have been observed with the cysts of Taenia pisiformis in rabbits. Rabbits infected with this parasite were resistant to subsequent feedings of oncospheres (Miller and Kerr). Kerr (1934 and 1935c), carrying out the work in detail, permitted the cysts from a first infection to become established in the mesenteries before feeding a second quantity of oncospheres. At autopsy, which was carried out at a time after the second feeding when the developing cysts were in the liver, marked resistance was shown by the previously infected rabbits, fewer cysts from the second feeding being present in the liver and these for the most part being poorly developed or dead.

Turner and associates (1935a) concluded that sheep probably become to some degree immune to the larval stage of Echinococcus granulosus, and the Penfolds and Phillips reported that a primary dose of 400,000 eggs of Taenia saginata conferred on oxen immunity against a similar dose at fifty-two and at seventy weeks after the initial infection. Penfold and Penfold found that as few as 5 Cysticercus bovis were sufficient to immunize cattle against a subsequent infection with the parasite. The cysts were found to remain viable only about nine months, although the immunity persisted for a long time thereafter. The authors suggested using the method of immunization by very low grade infection in Syria and other regions where the disease is prevalent.

The work of Hunninen (1935) with Hymenolepis fraterna in mice showed that infected animals resist both natural and artificial reinfection, as shown by the failure of cysticercoids to develop following administration of oncospheres to mice already infected. This immunity sometimes follows an initial infection from feeding as few as from 250 to 500 viable oncospheres and is found to persist in some mice for as long as eleven months. The same author (1936) reported that normal mice which are infected with Hymenolepis fraterna are not susceptible to internal autoinfection with this parasite and suggested that internal autoinfection does not occur in otherwise healthy human beings who harbor Hymenolepis nana.

(*c*) Nematoda. Many nematodes, in the course which they take through the blood and tissues in their development, can be expected

commonly to induce immune responses in the host. These responses may completely or in part protect the host from reinfection or may limit the size, the persistence and the egg-laying propensities of the parasites after they have succeeded in establishing themselves.

Several studies on immunity to infection with ascarids have appeared. Graham, Ackert and Jones noted that in 84 chicks infected with Ascaridia lineata at 5 and again at 10 weeks of age the number and size of the worms from the second feeding indicated that slight immunity resulted from the earlier feeding. They suggested that the basis of the immunity might lie in the antibodies produced in response to the invasion of tissues by the parasites. Sarles and Stoll noted that the cat is resistant to superinfection with Toxocara cati. On the other hand, Morgan (1931b), as well as de Boer, reported that previous infection of pigs with Ascaris lumbricoides did not prevent superinfection, one of Morgan's two previously infected animals succumbing to the superinfecting dose.

McCoy (1931b) tested the response of dogs and cats to repeated infection with infective larvae of Ancylostoma canium, which were fed to the animals in gelatin capsules. He concluded from the results obtained after feeding the test dose to the experimental animals and the controls that in all cases, even of light infection, a specifically acquired immunity distinct from the immunity acquired with age resulted. In no case, however, was there natural disinfection as a result of this response, although the egg production was reduced to about a third of the generally accepted normal figure. Foster (1935), on the contrary, reached the conclusion that no immunity results specifically from the infection in dogs. Sarles (1929a) found that dogs which harbored hookworms for long periods were more resistant to reinfection but that the resistance was chiefly due to age, only a minor part being accredited as a specific response to infection. Herrick previously had concluded that in dogs no increase in resistance followed an infection cured by anthelminthics, although in those cases in which worms remained from prior infections enhanced resistance to superimposed infection was evident. Recently Otto, Kerr and Landsberg reported that young dogs were protected against overwhelming infections with Ancylostoma canium by from 25 to 30 immunizing infections with doses gradually increasing from 15,000 to 120,000 larvae. None of the 4 immunized dogs showed appreciable symptoms from the test infections. Two that were killed and examined three weeks after infection with 120,000 larvae yielded only 118 and 203 hookworms, respectively, compared with 13,400 worms recovered from a control dog which died on the ninth day, and 2 immunized dogs of another litter given 2,000,000 larvae for a test dose yielded 52 and 56 worms after thirty-five days, compared with 1,263 worms recovered on the thirty-

fifth day from a control dog. These workers pointed out that a protracted period of treatment is necessary to immunize effectively against the hookworm.

Kerr (1935b and 1936) studied the resistance of mice to larvae of Ancylostoma canium. He noted that generally mice died in seven days after infection by mouth or through the skin with 2,500 larvae but that mice given sublethal doses of larvae either by skin or by mouth resisted a dose of larvae well above the average lethal number. Fifty-six mice which had been given weekly doses increasing geometrically from 250 larvae tolerated the fifth dose, 4,000 larvae, while the control mice, with an injection of 4,000 larvae, died in four days. When 8,000 or 16,000 larvae were administered, however, even those with previous injections succumbed. In another experiment, 5 mice which had been infected with 1,500 larvae resisted infection with 4,000 larvae four weeks later. In a third experiment, 5 mice were exposed to 50 larvae at weekly intervals for five weeks. Two of these animals were able to resist infection with 4,000 larvae. Kerr (1936) referred to the advantage of using an abnormal host. He pointed out that the larvae pass through tissues in the normal manner but that the parasites do not become established in the intestine. In the development of immunity, therefore, the importance of the larval stages, in which there is more intimate contact with the tissues of the host, can thus be determined selectively.

A marked response of immunity to the rat nematode, Nippostrongylus muris, has been noted by several workers. Africa and Graham found that previously infected rats became completely or partially refractory to subsequent infection, particularly if the initial infection was heavy. Africa noted also that disinfestation occurred earliest in those animals which were most heavily infected. Chandler (1932a) obtained more limited evidence of immunity following repeated infection of rats with Nippostrongylus muris, although he found this to be specific so far as it went. He (1935f) reported that the greatest resistance of rats to Nippostrongylus infection occurred in from fourteen to fifteen days after the first feeding and that after thirty days it was so reduced that a high percentage of invading larvae become adults, although these adults persisted in the intestine but for a short time.

McCoy (1931c) tested the immunologic response of rats and monkeys to Trichinella spiralis. As a result of repeated administration of living larvae, rats were usually found to manifest marked resistance to superinfection with this parasite, generally surviving more than twice the dose of larvae which proved lethal to the majority of the control rats. For the immunizing infections, doses of from 500 to 8,000 larvae (the number being doubled each second week) were given by stomach tube at weekly intervals. Three weeks after the last administration, these infected rats together with the control rats were each given by stomach tube a test dose of 70 larvae per gram of body weight. One of the 11 experimental rats succumbed to the test dose, whereas, of the 11 control rats, 9 succumbed. Three rats showed, on examination of the intestines, that only 0.04, 0.05 and 2.8 per cent, respectively, of the larvae of the test dose developed, in comparison with 35, 28 and 35.2 per cent, respectively, in the same number of control rats. Likewise, by digestion of the muscle of 3 immune rats, 8,400, 1,950 and 1,750 larvae, respec-

tively, were recovered, whereas from the same number of controls 570,000, 535,000 and 1,527,000 larvae were obtained. These figures show conclusively that in rats marked resistance to superinfection follows on infection with Trichinella spiralis. McCoy (1932) found, however, that monkeys do not so regularly become resistant to reinfection with this parasite after previous infection. Bachman and Oliver Gonzalez (1936) were also able, by feeding small and gradually increased doses of trichinous meat, to confer protection on rats against a lethal dose of meat subsequently given.

Marked resistance to superinfection with Strongyloides ratti is acquired by rats from an existing infection with this parasite. If an infection is let run its course until it disappears, almost absolute resistance is acquired. The resistance persists for at least sixty-eight days after the initial infection has disappeared, as shown by the low percentage of subsequently injected larvae attaining full development. The refractory state can also be attained if the rats are given injections of heat-killed larvae (Sheldon).

Other species of nematodes have been employed for immunologic studies. Rabbits show high resistance to Trichostrongylus calcaratus between the sixth and eighth weeks of infection and not only are thereafter refractory to reinfection but discharge worms harbored from the initial infection (Sarles, 1932). Kauzal expressed the belief that the resistance of lambs to superinfection with Dictyocaulus filaria is in part accounted for by specifically acquired immunity. Schwartz and Lucker studied specifically the superinfection of pigs with the lungworm Metastrongylus elongatus. Four pigs 70 days old were infected with 2,500, 1,500, 1,000 and 500 larvae, respectively, which had been reared in earthworms (Helodrilus). The total egg production by the worms in each pig was inverse to the number of larvae, and the patent period in those given the larger dose was decidedly shorter. After egg production ceased in all animals, they were reinfected with larvae of Metastrongylus elongatus and of the closely related form Choerstrongylus pudendotectus. Forty-five days later the pigs were killed and examined. The parasites recovered from the two pigs given the large initial infections were nearly all immature and no female contained eggs, whereas the pigs given the smaller doses harbored sizable infestations and many females of both species contained eggs in various stages of development. Taylor held that lambs placed on infective pasture (Haemonchus contortus, Nematodirus filicollis) early in life acquire immunity to parasitic gastritis by the eighteenth week and that this assists the animals to withstand repeated reinfection under conditions which result in death of lambs not so protected in less than seven weeks after infection. Stoll (1936) stated that this immunity is maintained by constant reinfection and that the heavier the infection is the greater will be the resistance. Ross, however, considered that acquired resistance, if produced, is of only short duration.

Artificial Immunization.—1. Preparation of the Antigen. In preparing antigens for use in artificial immunization, helminths are sometimes extracted with various solvents, such as sodium chloride solution, 95 per cent alcohol and Coca's solution; these extracts are then administered.

Often, on the other hand, the helminth is merely dried and powdered and then suspended in these fluids for injection.

(a) Trematoda. Kerr and Petkovich injected a 1 per cent saline solution suspension of dried adult Fasciola hepatica.

(*b*) Cestoda. Miller (1930) and Kerr (1935c), in their work with Taenia taeniaformis and Taenia serrata, used as antigen powdered adult worm material. Ohira employed emulsions of larvae of Cysticercus fasciolaris as antigen. Campbell (1936a) studied the antigenicity of various fractions of both the adult and the larval form of Taenia taeniaformis. He found that the whole worm and the globulin, nucleoprotein, saline-soluble or saline-insoluble, and albumin fractions of either the adult or the larval form all produced high resistance. The albuminoid and polysaccharide fractions also stimulated resistance of varying degrees, but the albumin from the dry worm and the metaprotein produced little, if any, protection.

The fluid of the hydatid cyst as well as that of other larval taenias serves admirably as antigen (Fairley, Fairley and Williams; Morenas, 1932). The scolices and membranes of cattle hydatids, dried at 37 C., powdered and made up in a 1 per cent suspension in 0.5 per cent phenol, also are useful as antigen (Turner, Berberian and Dennis, 1933).

(*c*) Nematoda. McCoy (1935) employed live larvae, larvae killed by heating at 55 C. for a few minutes, and suspensions of powdered dry, larvae of Trichinella spiralis as antigen in his artificial immunization of rats against Trichinella spiralis. Wagner used non-embryonated eggs of Ascaris lumbricoides for immunizing mice against later infection by embryonated eggs. Canning studied the reactions of antiserums prepared by injecting antigens coming from single organs or single germ layers of Ascaris, using for the purpose eggs, sperm, muscle, intestine and cuticle. Campbell (1936b) studied the reactions of a polysaccharide which he derived from Ascaris and which has antigenic power independent of the proteins of the parasite.

2. Response in Different Groups. (*a*) Trematoda. In one of the very few papers on artificial immunization against trematodes, Kerr and Petkovich reported partial immunization of rabbits against Fasciola hepatica by injecting a 1 per cent suspension of dried sheep liver flukes in saline at intervals of from one to two days over a period of three weeks. The immunized animals showed evidence of calcification of the flukes and passed no eggs in their feces.

(*b*) Cestoda. Miller (1930) successfully immunized rats against oncospheres of Taenia fasciolaris by injecting intraperitoneally powdered dried, worms at from two to three day intervals for five weeks. When the rats were killed and examined one month after the test feeding of oncospheres, most of the cysts which had developed were dead, and these had attained a diameter of only 1 mm. Control rats, fed the same num-

ber of oncospheres, showed many live cysts from 2 to 5 mm. in diameter. The specific character of the antigen is apparently an important consideration, since rats immunized with Taenia pisiformis, a closely related form, did not manifest resistance against oncospheres of Taenia fasciolaris (Miller, 1931c). The immunity of the rats had developed by the day following the last of the immunizing injections, and it persisted for over five months. Injections begun after infection with the oncospheres, however, did not inhibit development of cysts (Miller, 1932h). Campbell (1936b) repeated some of the work of Miller, with the same results.

An essentially similar manifestation of immunity was pointed out by Kerr (1935c) in rabbits given injections of material from Cysticercus pisiformis. In autopsies on the rabbits from three to six weeks after the test infection, he (1934) found that often complete resistance had been conferred by the earlier injections of antigen.

Some of the studies on immunity against the intestinal, or adult, form of cestode have yielded promising results. Ohira reported that kittens are rendered partially immune to Taenia crassicollis by subcutaneous injection or feeding of emulsions of Cysticercus fasciolaris. The adult worms present in the intestines of the treated kittens after a test feeding with cysticerci were smaller and fewer and matured more slowly than those in controls. Somewhat similar results have been obtained in puppies against Diphyllobothrium latum (Ohira). Dogs have been immunized against Taenia echinococcus by an antigen consisting of powdered dried hydatid scolices and membranes injected five times at from three to five day intervals. When fed fresh fertile cysts from six to fifteen days after the last of the immunizing injections, the dogs remained largely free from the parasites, whereas control dogs became intensely infected (Turner, Berberian and Dennis, 1936; Turner, Dennis and Berberian, 1935a). Recently these investigators succeeded in obtaining an increase of resistance against hydatids in sheep. While the procedure used did not prevent infection, fewer cysts developed in the treated animals than in controls and the cysts were walled off more readily and more efficiently than those in the controls. The sensitization of the treated sheep through prior contact with the hydatid antigen apparently permitted them to react earlier and more intensely than normal animals, the reaction being of the character of an Arthus response (Turner, Dennis and Berberian, 1937).

Dévé (1934), however, was unable, by 5 weekly injections of killed hydatid material, to immunize mice against a subsequent injection of living hydatid sand. In rabbits, also, Dévé (1934) failed to demonstrate immune responses effective in the prevention or cure of the infection.

Mueller and Chapman immunized rhesus monkeys against Diphyllobothrium Mansonoides with 9 injections of 0.5 per cent antigen in distilled water, the dose being graded from 0.1 to 4 cc. Experimental and control animals became infected equally following a test dose of the parasites, although the larvae in the immunized animals were walled off in tough cysts, especially in the regions where the antigen had been injected, whereas in controls the larvae were found mainly free in the tissues.

(*c*) Nematoda. McCoy (1935) showed that rats can be immunized against Trichinella spiralis by injecting intraperitoneally live larvae or,

less efficiently, larvae killed by heating at 65 C. for five minutes or larvae which have been dried and powdered. Five rats that had received 6 intraperitoneal injections of from 2,000 to 8,000 live larvae at five day intervals, together with 5 control rats, were fed, 2,000 live larvae by stomach tube fifteen days after the last immunizing injection. The ratio of the number of larvae that developed (recovered by digesting the muscle five weeks later) to the number of larvae fed was in the inoculated rats 8.1 and in the controls 24.9. When this experiment was repeated, 6 intraperitoneal injections being given, each of from 5,000 to 10,000 living larvae, the ratio was considerably more favorable as indicating that an immune response had occurred, only 1.3 larvae being recovered from the experimental rats for each larva fed, and 56.1 larvae being recovered from the controls. In another experiment, only heat-killed larvae or larvae which had been dried and powdered were used as antigens. Eight rats were each given 6 intraperitoneal injections of 5,000 heat-killed larvae, 6 rats were each given 6 intraperitoneal injections of 0.05 Gm. of dried trichinella powder, and 8 rats were kept as controls. Three weeks after the last inoculation, each rat was fed 2,000 live larvae by stomach tube. The ratio of the larvae recovered from muscles five weeks later to those fed was in the rats given heat-killed larvae 52.3, in those given dried powder 59.3, and in those kept as controls 107. When nonspecific worm substance (Taenia saginata) was used as the antigen, no evidence of an immune response by the rats was demonstrated. While the results obtained by McCoy (1935) with heat-killed larvae and dried, powdered larvae are at variance with those reported by Bachman and Rodriguez-Molina (1933), who concluded that prior immunization of hogs over a period of twenty days did not confer immunity against Trichinella spiralis, the figures of McCoy are convincing, and there remains little room to doubt that with delicate technic an immune response to Trichinella can be detected in at least the rat.

Spindler (1937) noted that metabolic products of Trichinella spiralis in muscles contain a substance capable of inducing some immunity to trichinosis when fed by mouth to rats, rabbits and guinea pigs. The substance is secured from trichinous meat by treatment with gastric juice and is stable to moderate heating.

Chandler (1932a) also brought forward substantial evidence that a specific immune response against Nippostrongylus muris can be developed by artificial means in rats. A total of 10,000 larvae, killed by heating at 55 C., were given in 5 injections at seven or eight day intervals. One week after the last injection, 250 live larvae were administered. Egg counts, begun on the eighth day following, showed in the inoculated rats an average of 500 and a maximum of 1,900 eggs per gram of feces and in the controls an average of 10,000 and a minimum of 5,600 eggs per gram of feces. The live worms recovered from the inoculated

and the control rats at autopsy from eleven to twelve days after the first egg counts showed no significant difference in numbers, but female worms from the inoculated rats were little more than half as long as those from the controls, and many were immature. The same author (1936h) found that ingestion of dead adult or larval worms had no appreciable effect on a subsequent infection.

Wagner found that inoculation of mice with 2 or more doses of the eggs of Ascaris lumbricoides or injection of powdered dried ascarids inhibited later infection, only few larvae from the later infection being able to migrate to the liver or lungs from the intestine.

Passive Transfer of Immunity.—1. Attempts in Different Groups. (*a*) Trematoda. So far as I have determined, no one has reported the results of experiments to test the possibility of a passive transfer of immunity to trematode infections except Nigrelli, whose work I have referred to. Nigrelli found that immune serum conferred on susceptible fish no immunity against the monogenetic trematode Epibdella melleni.

(*b*) Cestoda. The serum of rats which are immune to Cysticercus fasciolaris inhibits the development of oncospheres fed to nonimmune rats. If the serum is injected intraperitoneally at the same time as the oncospheres are fed, almost complete inhibition can be expected, but if it is given nine days or more subsequent to feeding the oncospheres, little inhibition of the larval development can be expected (Miller 1932f and 1934). The serum can come from rats infected with the parasite or from rats or rabbits artificially immunized against the parasite by intraperitoneal injection of a 1 per cent suspension of powdered tapeworm material (Miller and Gardiner, 1932).

In an experiment by Miller and Gardiner, 12 rats given serum from infected donor rats, 12 given serum from artificially immunized donor rats and 12 controls were fed 900 oncospheres each. After thirty-seven days, at autopsy, the animals of the first lot showed no infection, those given serum from artificially immunized rats showed on the average 0.5 live and 6 dead cysts per rat, and the control rats showed 22 live and 19 dead cysts each. These results indicate not only that the immunity to this parasite can be controlled but that the serum obtained from an infected rat is more potent in its protective action than that from an artificially immunized animal (Miller and Gardiner, 1932c). The immunity thus conferred on normal rats persisted for at least twenty-six days, and in 2 of 4 animals reported on by Miller and Gardiner (1932c) was present after thirty-six days.

In order that serum from an infected animal may be protective to another rat into which it may be injected, the donor rat should have been infected during at least ten days. In most cases, serum from donors infected for ten days or more inhibit completely the cyst development in the recipient rat if given in a quantity of 1 cc. or more per five hundred and twenty-five grams of rat. The serum of donors that have been infected from four to ten days also depresses to some extent the number of cysts which develop, although complete inhibition of their development is not obtained (Miller and Gardiner, 1934d).

Kerr (1934 and 1935c) showed that the serum of rabbits infected with Taenia pisiformis or artificially immunized with the material of this parasite confers immunity on other rabbits into which it is injected. For example, 2 cc. of serum per hundred grams of body weight of the recipient rabbit prevented entirely the

development of cysts in 4 of 8 experimental rabbits. At autopsy, twenty-one days after feeding 900 oncospheres, the average number of live cysts developed in 7 of the experimental rabbits was 1.16, compared with 14.16 in the control rabbits.

In contrast to these results, Dévé (1933) failed to obtain protection of mice against Taenia echinococcus "sand" by injecting the serum of a street dog infected with various taenias.

(*c*) Nematoda. Chandler (1934 and 1935e) obtained no evidence that the immunity which he (1932a and 1935f) demonstrated in rats against Nippostrongylus muris could be transferred to normal rats. Immune serum was obtained from rats which were repeatedly inoculated with larvae of Nippostrongylus muris at from seven to fourteen day intervals till a high degree of immunity to reinfection was attained. Normal serum, which was injected into one set of control rats, was obtained from uninfected rats of comparable age. The rats in which the test of passive transfer of resistance was carried out were from 3 to 6 months old and therefore too young to show age resistance. The test doses of larvae consisted of from 250 to 300 infective larvae from five to eight day cultures. In searching for evidence that the normal rats had been rendered resistant by an injection of the serum from the immune animals, Chandler (1) made egg counts from the seventh to the eleventh day after infection to detect any differences in the prepatent period or in the nature of the rise to the peak, (2) killed and examined half the rats after from ten to fourteen days to determine the maximal number of worms produced at the height of the infection, (3) carried out egg counts on the remaining rats from the fifteenth to the twenty-fifth day to detect any differences in the time and extent of falling off in the egg production and (4) made final autopsies to discover differences in the rate of loss of worms. The results indicated that the immune serum had no effect on the length of the prepatent period, the maximal egg production, the time required to reach the peak of egg production, the average number of eggs produced by each female, the time of falling off of egg production or the number of worms present after fifteen or twenty-five days.

Chandler (1934 and 1935e) showed further that passive transfer of immunity against this parasite does not occur with the use of parabiotic twins, one member of the pair being immunized, before or after union, by a series of injections each of several hundred larvae at intervals of one week, a total of from 1,000 to 3,400 worms being given. For the test infection, 300 larvae were administered. A count of the number of the worms found at autopsy nine days after the test infection revealed no evidence that the actively immunized member of the pair conferred on its twin resistance to the parasite. From the results of these two approaches to the problem Chandler concluded that the blood stream is not involved in any of the various phases of immunity which actively immunized rats manifest against Nippostrongylus muris. The recent work of Sarles and Taliaferro has tended to show, however, that the immunity of rats against Nippostrongylus muris can be passively transferred to normal animals, in the serum. These workers were able to elicit protection by the transfer of 4.1, 4.5 and 6 cc. of hyperimmune

serum per hundred grams of rat to rats simultaneously infected percutaneously with from 3,500 to 4,000 larvae.

From the experiments on mice infected with Ancylostoma canium, Kerr (1935b) was unable to show that the resistance which he demonstrated could be passively transferred.

Trawinski (1935) reported that from twenty-five to thirty-five days after rabbits have been heavily infected with Trichinella spiralis they yield a serum which protects rats against several lethal doses of the larvae. Although rats which were given 3 cc. of the serum five days before the test infection with larvae died from ten to seventeen days after injection, 92.6 per cent of those given 3 cc. of the serum ten days after infection with his lethal dose (200 larvae) remained alive for five weeks. When the therapeutic tests were repeated, with use of more than 3 lethal doses of larvae, similar results were obtained. At autopsy, at the end of the five week period of observation, the muscles of the successfully treated animals were found heavily infected with the larval parasites. It is suggested from this that the serum is antitoxic rather than antiparasitic in its mode of action.

The possibility of passive transfer of immunity in experimental trichiniasis has also been investigated recently by Culbertson and Kaplan. These workers noted that the serum of rabbits heavily infected with Trichinella spiralis shows after about eight weeks a considerable concentration of antibody. When this serum is injected into mice which are fed from 175 to 300 larvae of this parasite, only about half as many of these larvae mature as mature in control mice not so treated. No effect of the serum on the muscle-invading larvae produced by the adult Trichinella in the intestine has been noted. It appears from this work that the potentialities of serum therapy in trichiniasis are small, since to be effective the serum must be given in the first two or three days after infected meat has been ingested, at a time prior to the appearance of symptoms of disease. It seems possible, however, that serum more potent in antibody against Trichinella may be developed which will affect the parasite in the somatic stages.

2. Attempts via the Placenta or the Milk. (*a*) Trematoda. No record has been found of work in which trematodes were employed.

(*b*) Cestoda. Miller (1932d and 1935k) was able to confer on rats considerable immunity to infestation with Taenia taeniaformis by infecting or by actively immunizing the mothers before these were mated. The greater resistance was seen in the offspring of the infected mothers. In artificial immunization better results were obtained by injecting oncospheres into the mothers than by treating them with powdered Taenia taeniaformis material. In most instances, cyst development was completely inhibited or nearly so in the offspring of the immune mothers, whereas on the average 35 living cysts from 2 to 5 mm. in diameter were present in the liver of each control rat at autopsy four to five weeks after feeding infective oncospheres.

Shorb noted initial resistance of rats and especially of mice to Hymenolepis fraterna during the nursing period, which is lost soon thereafter. This may also be evidence of the transmission of resistance to a cestode parasite either congenitally or through the milk.

(*c*) Nematoda. Chandler (1934) reported on the transfer of immunity to Nippostrongylus muris from mother rats to offspring. The details of the experiment are not available in the abstract cited.

II. CONDITIONS OR AGENCIES WHICH AFFECT RESISTANCE

INTERCURRENT INFECTION

(*a*) Trematoda. No experimental studies on the effect of intercurrent infections on resistance to trematodes have appeared.

(*b*) Cestoda. Hunninen (1936) reported that, although internal autoinfection with Hymenolepis fraterna does not occur in normal mice, this mode of infection may occur in mice in which natural resistance has been decreased by some factor, such as bacterial disease. In mice suffering from what was probably mouse paratyphoid, it was noted that cysticercoids developed in the posterior part of the intestine, presumably as the result of infection from eggs of adult worms present further forward in the intestine. Such infections were not observed in normal mice.

(*c*) Nematoda. Baker showed that in the presence of blackhead there are retardation of the development of, and reduction in the number of, Heterakis gallinae as compared with what one finds in healthy birds. Elsbach noted that Javanese and older British Indians and bushmen in Paramaribo escape clinical filariasis, whereas young British Indians and creoles in the towns suffer, although the exposure of all to infection is the same. He explains this difference by a highly developed resisting power on the part of the reticulo-endothelial system due to many malarial attacks in the former and by the absence of this stimulus in the latter.

DIET

(a) Trematoda. No work on the relation of diet to the susceptibility of animals to trematodes has been recorded.

(*b*) Cestoda. Shorb reported that a diet of white bread and water reduces the resistance of rats to nonspecific strains of Hymenolepis fraterna and also the resistance which is connected with age. On the other hand, the worms will not develop to maturity in mice on this diet, suggesting either that the resistance of mice on such a diet is enhanced or that some factor which is directly essential for the maturing of the parasite is absent from the food.

(*c*) Nematoda. Foster and Cort showed in a series of studies that in dogs a definite correlation exists between undernourishment and susceptibility to infection with Ancylostoma canium. Through undernourishment, dogs in which high resistance to infection had developed with age or through previous infection became less resistant, as shown by increase in the rate of development of the worms, in the egg production of females and in susceptibility to reinfection. On transfer of the animals from a deficient to an adequate diet, the natural resistance of the dogs was recovered, as shown by spontaneous loss of the worms present, by reduction in egg production and by resistance to further infection (Foster and Cort, 1931 and 1932). Certain animals showed a terminal complete breakdown of resistance, resulting in a sharp increase in egg production and death of the dogs. In general, young animals proved more susceptible than older dogs to the effect of a deficient diet (Foster and Cort, 1935). Cort (1932) suggested that diet may similarly influence the resistance of man to the hookworms that infest man and that this resistance may be broken down if the diet is poor in quality. Children were found by Ahmann and Bristol to show a 100 per cent decrease in the ascarid burden after being put on an adequate diet, and some tendency toward reduction of the hookworm infestation as well, determinations being made in both cases by egg counts according to the method of Stoll.

Chandler (1932a) noted no significant difference in the resistance of rats to Nippostrongylus muris as a result of a deficient diet if the animals were not seriously injured by the deficiency. More worms were recovered from rats fed on coarse vegetables and fruit till they were emaciated and unthrifty than from rats on a normal diet. Porter (1935b) found that rats fed on whole milk showed markedly less resistance both to primary infestation and to reinfestation with Nippostrongylus muris.

The resistance of sheep to Haemonchus contortus is also adversely affected by a deficient diet (Ross) if the deficiency is sufficiently severe to cause loss in weight (Ross and Gordon); in some cases, nutritional factors can not be shown to influence resistance. Fraser and Robertson reported that well fed lambs picked up an average of 31 worms in contrast to 103 worms picked up by poorly fed lambs pastured on the same ground. The well fed group was given 3 pints (1.4 liters) of separated milk and 1 pound (373 Gm.) of mixed meals daily, whereas the poorly fed group received from 7 to 8 pounds (2.5 to 3 Kg.) of green tares and bruised vats.

Kauzal noted no change in the susceptibility of lambs to Dictyocaulus filaria as a result of a deficient diet, even when there was a concurrent infection with Haemonchus contortus.

Ackert (1935) reported that the resistance of chickens to Ascaridia lineata was affected by diet, a purely vegetable diet seldom leading to strong resistance against this parasite (Ackert and Beach).

Vitamins.—(*a*) Trematoda and Cestoda. I have found no work on the influence of vitamins on susceptibility and resistance to trematodes or cestodes except that

by Seifried, who concluded that fowls were rendered more susceptible to Davainea proglottina by a diet deficient in vitamin A.

(*b*) Nematoda. In the case of several nematodes, a deficiency of vitamin A in the diet has been shown to lower resistance to infection. McCoy (1934) reported that albino rats on a ration deficient in this vitamin are less resistant to Trichinella spiralis by the second week of the diet. The diminished resistance is shown by the longer persistence of adult worms in the intestine and by the larger number of larvae which migrate and settle in the muscles. Furthermore, rats fed on such a diet exhibit no resistance against a second infection with the parasite. A similar though less marked effect of the lack of vitamin A is seen as well in older rats.

Spindler (1933) noted lowered resistance against Nippostrongylus muris in rats fed a diet deficient in vitamin A, both to initial infection and to reinfection. He observed in rats on a vitamin-free diet and infected for the first time with Nippostrongylus muris patent periods of from sixty-four to sixty-seven days as contrasted with periods of from eleven to fourteen days in controls fed a complete diet. The egg counts in the experimental rats reached 41,000 as compared with 250 in the controls, and at autopsy, on the average, 5 larvae were recovered from the lung and 139 female worms from the intestine in contrast to 68 larvae from the lung and 7 female worms from the intestine of each of the controls fed a full diet. In animals exposed to superinfection, patent periods of thirty-one days and of from twenty to twenty-one days, respectively, were observed in experimental and control rats. Egg counts reached 2,600 in the experimental and 1,500 in the control animals. At autopsy after superinfection, on the average, 5 larvae were found in the lung and 60 females in the intestine of each rat fed the vitamin A-free diet, compared with 27 larvae in the lung and 17 female worms in the intestine of each control.

A number of papers have appeared on the effect of vitamin A on resistance to ascarids. Some have reported that vitamin A deficiency is without effect on the susceptibility of pigs to the human or pig ascarid (de Boer; Clapham, 1934; Mohler), although Kurisu concluded that lack of this vitamin predisposes rats and pigs to ascariasis. The susceptibility of dogs from 2 to 4 years of age to Toxocara canis is apparently increased when they are fed on a vitamin-free ration (Wright, 1933; Mohler). Wright (1935) recovered 2,674 Toxocara canis and Toxascaris leonina, or 243.1 worms each, from 11 dogs from 82 to 154 days old which were fed on a diet free or partly free from vitamin A for from fifteen to one hundred and six days. Nine control dogs of similar age and fed a complete diet harbored 488 worms, an average of 49.8 worms per dog. Absence of vitamin A from the diet is said to affect also the number and the rate of development of Ascaris equi in rats (Clapham, 1933). Ackert (1932) and co-workers, as well as Seifried, showed that certain vitamins are important in the resistance of chicks against Ascaridia lineata. Without vitamin A, the size and number of worms are greater than in controls (Ackert, McIlvaine and Crawford). Without vitamin B, more worms develop, but their size is less than in control animals to which baker's yeast is fed as a source of vitamin B (Ackert and Nolf). In most experiments, vitamin D was without influence on the resistance of chicks to Ascaridia lineata (Ackert and Spindler).

Clapham (1933) noted no effect of vitamin A on the resistance of chickens to Heterakis gallinae, although chicks 10 weeks old could be rendered susceptible to Syngamus trachealis by feeding on a diet deficient in vitamin A (Clapham, 1934d).

Specific Chemicals.—(*a*) Trematoda and Cestoda. The influence of specific chemicals on susceptibility and resistance to trematodes and cestodes has not been investigated.

(*b*) Nematoda. Spindler (1933) found that administration of ferrous sulfate and copper sulfate (560 mg. of each in 1 gallon [about 4 liters] of water) to pigs favored the development of larvae of Oesophagostoma dentatum which were fed to these animals. Following infection, the pigs were killed, and the nodular worms were counted. Although there were more worms present in the pigs to which the salts were administered, these animals seemed to thrive better, perhaps because of some stimulating effect of the salts on the hosts.

Clapham (1934d) reported that 10 week old chicks, which are normally resistant to Syngamus trachealis, become susceptible to infection with this parasite if their diet is deficient in calcium as well as in vitamin A. Graham, Ackert and Jones believed that administration of carbon tetrachloride injures the liver and causes the ionized blood calcium to unite with bile, leading to retardation in the growth of young worms unless additional calcium is fed.

Dogs and cats given a milk diet become susceptible to infection with hookworm by reason of the anemia induced by the deficiency of iron in milk (Foster, 1936b).

PREGNANCY AND REPRODUCTION

The influence of pregnancy on resistance to helminth infection has been little studied. Chandler (1932b) suggested that pregnancy may alter the natural resistance to infection, and Hunninen (1935) reported that in two or three days after delivery the female mice with which he was working gave no evidence of infection with Hymenolepsis fraterna. The immunity of sheep to Haemonchus cortortus is reduced in rams in the breeding season and in ewes during reproduction and especially during lactation (Stoll, 1936).

HEMORRHAGE

The influence of acute hemorrhage on resistance to a specific parasite has been studied in some cases (Ackert, 1935). Porter and Ackert found that repeated loss of blood lowers the resistance of chickens to Ascaridia lineata. More and longer worms were usually obtained from birds bled repeatedly during the course of infestation. Cort (1932) and Foster (1936) pointed out that the mechanism of resistance to the hookworm in dogs is sometimes broken down by repeated bleeding.

III. BASIS OF IMMUNITY AGAINST PARASITES

EFFECT ON THE PARASITES

The immune responses which the host makes against parasites which invade it affect the parasites directly in a number of ways. The actual number of helminths which develop following administration of an infecting dose is less in the immune animal than in the normal animal, the former sometimes manifesting complete resistance to infection. When the parasites are able to develop in the immune animal they are often of smaller size than in the normal animal and give evidence either of retardation of development or of an inability to reach full maturity. When worms in the immune host produce eggs these are often significantly less in number than those in the normal host. These different effects on the parasite may be dependent on substances which develop in the host after infection and which are directed more or less specificially against the invader. These various subjects will be taken up in some detail.

Number of Parasites.—(*a*) Trematoda. Ozawa immunized dogs against Schistosomum japonicum in three ways: by infecting them, then curing them with antimony sodium tartrate, by injecting a suspension of adult worms and by injecting a suspension of cercarias. Not only were the symptoms which followed subsequent infection milder, but also fewer worms were able to develop, than in control animal, not previously immunized, and the worms showed very poor growth.

(*b*) Cestoda. A sharp reduction in the number of worms which develop after an animal has been immunized specifically has been demonstrated in regard to several cestodes. Rats immunized either by infection or by artificial means often inhibit entirely the development of the parasite when it is subsequently administered (Miller, 1931b and c). Similarly, complete immunity against Cysticercus pisiformis has been demonstrated in rabbits (Kerr, 1934a and 1935c). Hunninen (1935) found that fewer cysticercoids of Hymenolepis fraterna develop in immune than in normal rats, and Ohira reported that fewer worms develop in kittens previously immunized to Taenia crassicollis by either feeding or injecting emulsions of the larval parasites than in control animals.

(*c*) Nematoda. The most satisfactory papers that I have found showing a reduction in the number of worms occasioned by specific immunization are those of McCoy (1931c). After 3 rats had been infected with Trichinella spiralis, only 0.04, 0.05 and 2.8 per cent of a test infecting dose of 70 larvae per gram of body weight were able to develop to adults, compared with 28, 35 and 35.2 per cent which developed in 3 control animals. From the muscles of these same immune rats, 1,750, 1,950 and 8,400 larvae were recovered by artificial digestion, compared with 535,000, 570,000 and 1,527,000 larvae from the controls (McCoy, 1931c). Comparable figures were obtained after artificial immunization (McCoy, 1934). Culbertson and Kaplan recovered a smaller percentage (17) of adult Trichinella from mice passively immunized with rabbit anti-Trichinella serum than from control mice (36 and 37 per cent) after feeding a standard dose (from 175 to 300) of infective larvae of Trichinella. Bachman and Oliver Gonzales (1934) reported

the interesting observation that Trichinella spiralis lost its virulence for an unnatural host, the rabbit, after five successive passages, since the strain died out after 5 feedings in these animals, whereas in rats the number of worms per gram of meat continued to increase and the worms retained their power to penetrate the muscles of new hosts after successive passages in rats over a period of from nine to eleven months. Alicata failed to duplicate these results; he found, on the contrary, no reduction in the virulence of the parasite after passage at thirty-two day intervals through 7 pairs of rabbits, since guinea pigs and rats were easily killed in four days when the usual lethal dose of larvae was administered. Alicata believed that Bachman and Oliver Gonzales (1934) did not allow sufficient time between passages to permit the full quota of larvae to reach the muscles of the rabbits and that therefore, with succeeding passages, fewer larvae were fed, the fifth feeding being entirely free from them. Spindler (1934) obtained no significant difference in the number of Nippostrongylus muris in control rats and rats to which adult Nippostrongylus muris had been administered by duodenal tube. Sarles (1929b) noted that a smaller percentage of Ancylostoma canium develop in old dogs than in young animals. Ackert (1932) and others found large numbers of worms in hosts fed diets deficient in vitamins A and B.

Size of Parasites.—Evidence that worms after development in immunized animals are smaller than those that have developed in normal animals has been presented. Ozawa noted that adults of Schistosomum japonicum in immunized dogs were slender and of diminutive size. Ohira found that adult Taenia crassicollis in the intestines of artificially immunized kittens were smaller than those in controls, and Miller (1930) found that when larval forms of this parasite developed at all in immune rats they were on the average only 1 mm. in diameter, compared with from 2 to 5 mm. in normal rats. The stunting of Nippostrongylus muris after development in immune rats was reported by Chandler (1936g). Culbertson and Kaplan noted no significant difference in length between adult female Trichinella spiralis from mice given rabbit anti-Trichinella serum and those from mice not so treated.

Ackert (1932) found that if a host's diet was deficient in vitamin A, this led to increased size in Ascaridia lineata, although a deficiency in vitamin B did not produce any such effect. Larger worms of this parasite are recovered from birds bled frequently during the infection (Porter and Ackert).

Rate of Development of Parasites.—Ohira found that Taenia crassicollis was slower to mature in immune kittens than in normal animals. Taylor reported that the immunity which develops in sheep against reinfection with Haemonchus contortus and Nematodirus filicollis exerts an inhibitory effect on the subsequent development of the worms.

Extent of Development of Parasites.—Ozawa spoke of the high degeneration and structureless appearance of Schistosomum japonicum in immunized dogs. Chandler (1932a) observed that after rats are repeatedly infected with Nippostrongylus muris the worms are retarded in development and tend to reach only the fourth larval stage. When, however, these stunted forms are transferred to normal animals, they resume their growth and attain normal development. It thus appears that the inhibition in development resulting from residence in an immunized animal is not permanent (Chandler, 1936g). Scott had noted previously that larvae of Ancylostoma canium remain immature in an insusceptible host but proceed in development if transferred to a susceptible one. Sarles noted that

infective larvae of Ancylostoma canium are largely retained in the skin of old dogs during the first twenty-four hours, whereas in young animals they pass readily on.

Egg Production by Parasites.—A number of workers have shown that the egg production of female worms is depressed if the host is immunized against the parasite. Hunninen (1935) observed egg-negative intervals, often for long periods, in mice infected with Hymenolepis fraterna, and when eggs appeared they were in smaller number than in nonimmune mice. McCoy (1931b) noted that in dogs infected with Ancylostoma canium the eggs produced per female worm in resistant hosts was $5,364 \pm 313$ in contrast to $17,190 \pm 1,117$ per female worm in nonresistant animals. Chandler (1932a) noted that the egg production of Nippostrongylus muris was decreased in immune rats but that it rose when the adults were transferred to a normal rat (Chandler, 1936g). Spindler (1934) also found more eggs produced by Nippostrongylus muris in control rats (from 3,000 to 98,000 eggs per gram of feces) than in rats previously infected with adults administered by duodenal tube (from 800 to 13,600 eggs per gram of feces). McCoy (1935) noted diminished muscle invasion by the larvae of Trichinella spiralis in immunized rats. Taylor observed diminished egg production by Haemonchus contortus and Nematodirus filicollis in immunized lambs. Higher egg production by Ancylostoma canium in dogs occurs after the animals are given a deficient diet (Foster and Cort, 1935), and more larvae of Trichinella spiralis migrate and settle in the muscles of rats fed a vitamin A-free diet than in controls (McCoy, 1934).

Persistence of Parasites.—No very satisfactory work has appeared on the relative persistence of helminths in immune animals in contrast to normal animals. Chandler (1932b) and others pointed out, however, that an immunity response is characterized by loss of worms already established and, more specifically, called attention to the fall in egg production soon after the peak has been reached in hookworm infestation, explained presumably by the coincident elimination of adult worms (Chandler, 1935d).

McCoy (1932) observed that the length of time that adult trichinas persist in the intestines of rats is somewhat in proportion to the number present. A heavy infestation appears to break down normal resistance, which ordinarily limits the life of the adult worms.

Growth-Inhibiting Substances (Antienzymes).—Chandler (1935e and f) considered that the immunity of the rat to Nippostrongylus muris is due to the presence of antienzymes specifically elaborated by the rat after infection, which counteract the enzymes secreted by the parasite, by which the latter is enabled to utilize the proteins of the host. The immunity is therefore directed against the nutritional capacities of the parasite. The author believes that the immunity is essentially local, the elaboration of antienzymes being a property of the intestinal mucosa. Chandler (1936g) showed that these antienzymes effect no permanent injury on the parasite since, on its transfer to a normal host, it is able to grow to maturity and to produce eggs in the normal manner.

Ackert, Porter and Beach described a growth-inhibiting factor which they believed explains the natural immunity of old chickens to Ascaridia lineata. Whether this factor is a fluid passed from the intestinal wall into the lumen or whether it is a deposit in the mucosa or whether simply the mucosa becomes tougher is unknown. These authors referred to the work of Carrel and Ebeling, who showed the presence of an active growth-inhibiting substance for tissues in culture in chicken serum, which is present in larger quantities in the blood of older chickens.

IMPORTANCE OF HUMORAL AGENCIES

There is a peculiar lack of attention among helminthologists to the possible role which antibodies may play in the resistance of animals to their parasites. There no longer remains doubt that infections with helminths lead to resistance against superinfection. And specific antibodies have been demonstrated in a wide variety of helminthic infections. Yet the vast majority of workers have not appeared to consider that a correlation exists between the presence of antibodies and acquired resistance. Graham, Ackert and Jones suggested that the actively acquired immunity of chicks to Ascaridia lineata follows antibody production. They pointed out that the "boring proclivity of the young A. lineata places them in intimate contact with the serous fluids of the intestinal wall where they give off metabolic proteins which engender antibody formation." Bachman and Rodriguez-Molina (1933) expressed the belief that the resistance which they demonstrated in pigs against superinfection with Trichinella spiralis is due in part to local retention of antibodies. Sarles and Taliaferro concluded that the finding of a precipitate about and within the bodies of worms in the skin and lungs of immune rats, together with the passive transfer of immunity to Nippostrongylus muris among rats, indicates that the immunity of rats to this parasite has to a large extent an antibody basis. Kerr (1936) noted that definite reactions of the Arthus type occurred in immune mice at the sites of penetration of the infective larvae of Ancylostoma canium in the skin or stomach. Reactions of the Arthus type have also been shown in the livers of previously immunized sheep when these animals were exposed to infection with hydatids (Turner, Dennis and Berberian, 1937). Sarles (1937) reported that serum from rats immune to Nippostrongylus muris caused the formation of a precipitate about and within larval worms of this species exposed in vitro to its action for periods of several days. Arnold and Duggan found that blood of rabbits taken six weeks after the last of a series of injections of saline suspensions of macerated Dirofilaria immitis exerted a lethal action on the microfilarias of this species.

Further and more detailed studies are essential in order that this problem may be more fully solved. It has been adequately proved that the proteins of helminths are analogous to the proteins of other forms in their capacity to serve as antigens (Canning; Campbell, 1936a). It remains to prove whether the antibodies produced against these antigens are essential elements in protection.

Distribution of Antibody in the Host.—Fairley and Jasudasan found the complement fixation antibody in the serum and in the pleural, peritoneal and pericardial fluids, as well as in inflammatory exudates, but absent from the cerebrospinal fluid. These workers considered that the intact choroid plexus proves

an insuperable barrier to the passage of helminthic antibodies unless there is definite pathologic involvement of the central nervous system. Van Hoof demonstrated the fixation antibody for onchocerciasis in serum and in synovial, cerebrospinal and edematous fluids.

Time Relations of the Antibody Responses.—(*a*) Trematoda. The most complete available work on the time relations of the antibody responses in trematode infections is that of Fairley and Jasudasan. These workers infected 34 goats with cercarias of Schistosomum spindale, and demonstrated the presence of the complement-fixing antibody in 7 of these animals in the first week, in 14 in the second, in 3 in the third, in 4 in the fourth, in 1 in the fifth, in 4 in the sixth and in 1 in the seventh week of the disease. A primary rise in the amount of this antibody in the serum occurred usually in the second week, apparently as the result of invasion by the cercarias, and a secondary rise was noted between the fifth and the ninth week, related probably to portal thrombi of defunct schistosomes. (See graph 6, p. 98, Fairley and Jusudasan). From the third to the twelfth month, a well sustained curve of fixation existed, and in the second year strong positive reactions were recorded. Fairley and Jasudasan expressed the belief that the positive reaction persists as long as schistosomes are present in the body, although rarely the serum of an animal harboring numerous living worms and eggs may give a negative result long after infection. (See graph 12, p. 102, Fairley and Jasudasan.) Fairley (1926), using goats, had shown that when in the course of ten days an animal was exposed to 100,000 cercarias the fixation antibody appeared by the second and never after the third week, but that if an equivalent number of cercarias were applied in smaller doses over a longer period—from fourteen to sixty-four days—the antibody was correspondingly later in development, sometimes not appearing until the fourth or fifth week. In a number of animals cured by intravenous medication with such chemicals as urea stibamine (an antimony compound), antimony and potassium tartrate and emetine hydrochloride, reactions became negative about four to twenty-eight weeks after the beginning of treatment. In some other animals, low grade positive reactions persisted eighteen weeks after treatment, in the absence of live schistosomes. The authors considered that the absorption of antigen derived from disintegrating worms embedded in thrombosed veins was probably responsible for the persistent positive results.

(*b*) Cestoda. No study on cestode infections has appeared comparable in completeness to that of Fairley and Jasudasan on schistosomiasis with respect to the evolution of antibodies. Miller and Gardiner (1934) showed that rats must be infected for about ten days before their serum will confer complete protection against oncospheres of Taenia taeniaformis on transfer to normal rats. The antibody per se was studied by Campbell (1936a), who gave rats intraperitoneal injections of whole adult or larval worms, as well as of globulin, albumin, nucleoprotein and polysaccharide fractions. However, he did not report the time relations of the responses.

(*c*) Nematoda. Bachman was able to detect the precipitin antibody against Trichinella spiralis as early as the fifth day after infection of rabbits, i. e., even before larvae had entered the blood stream and when the adults were in the intestine. Culbertson and Kaplan failed to demonstrate precipitin in rabbits fed from 60,000 to 300,000 larvae of Trichinella spiralis when animals were bled after two weeks, although

all of 5 animals bled at four weeks gave positive results. In their tests on serum taken after eight weeks, more antibody was found than in earlier samples. Unfortunately no later samples were tested. Bachman and Menendez followed the complement fixation antibody in 8 rabbits infected with Trichinella spiralis. The titers rose from the third to the ninth day after infection, dropped from the fifteenth to the twentieth and rose rapidly again from the twenty-fifth to the thirty-fifth day. Blackie reported that rabbits infected with embryonated eggs of Ascaris megalocephala showed maximal fixation of complement on the fifteenth day after infection, 18 minimal hemolytic doses of complement being fixed at this time (see graph of Blackie).

IMPORTANCE OF CELLULAR AGENCIES

Excepting the fact that in many helminth infections the eosinophils of the blood increase markedly in both relative and absolute percentage, little is known concerning the role of the blood and tissue cells in protection against helminth infection. Taliaferro (1934) stated that the same kind of cells are involved in the animal response to an invasion by helminths as in that to an invasion by protozoa, bacteria or any foreign substance. The cells are those of the connective tissue and blood. The polymorphonuclear leukocytes are mobilized first, but the macrophages, which appear later, are chiefly responsible for overcoming the invader. As they may be needed, small lymphocytes can be transformed to macrophages. In acquired immunity, macrophages are of enhanced importance and may themselves produce weak antibodies. In helminth infections, the eosinophils are always prominent and probably are associated with defense against foreign toxic proteins, which they may actually be able to absorb directly.

Taliaferro and Sarles (1937b) took the point of view that acquired immunity is largely based on the action of antibodies, although during later stages certain cells function in clearing up parasites. These workers have indicated that antibodies are responsible for the immobilization of the larvae of Nippostrongylus in the passage of the invaders through the skin of immune rats, the larvae being surrounded by loose accumulations of cells or nodules comprised of eosinophils and hypertrophying agranulocytes. In the lungs monocytes which rise from local septal cells form nodules about the parasites in immune animals, and in the intestines of immune rats connective tissue, mast cells, eosinophils and macrophages increase immediately in the lamina propria, whereas in normal rats an interval of ten days after infection is required for this response.

Kerr (1935b and 1936) recently showed, in agreement with Taliaferro's point of view, that the macrophage cells of mice manifest a more significant defensive action than do the microphages against invasion by the larvae of the dog hook-

worm. Stumberg (1932) noted that repeated infection of dogs with Ancylostoma canium produced a graded fibrocyte proliferation in the dermis, but he thought that the histologic observations were consonant with mechanical injury by the larvae rather than with immunity reactions. Round cell infiltration occurred in no case. Sarles (1929b) noted that old dogs gave a more marked reaction than young dogs to the penetration of the larvae of Ancylostoma canium, the former showing marked and prolonged inflammation, compared with a slight and transient reaction or even absence of reaction in the young dogs.

Bachman and Rodriguez-Molina (1933) considered that leukocytes and cells of the reticulo-endothelial system supplement the action of antibodies to protect hogs from superinfection by Trichinella spiralis.

Blackie (1930) noted that in rabbits, guinea pigs and mice infected with larvae of Ascaris megalocephala the lungs showed intra-alveolar hemorrhage, with clusters of small round cells, eosinophils and macrophages, often enclosing a larva, and the liver showed clusters of cells consisting chiefly of eosinophils at the periphery of the lobules.

LOCAL IMMUNITY

Nigrelli and Breder observed that the moonfish (Vorner setipinnis) acquires a localized skin immunity to the ectoparasitic monogenetic trematode Epibdella melleni. When the fish, which had previously proved resistant, were handled, they became infected, but reinfections occurred only at new sites on the skin. Stumberg (1932) failed to demonstrate a significantly enhanced retention of the larvae of the dog hookworm in the skin of dogs previously receiving intracutaneous injections of hookworm antigen or antihookworm rabbit serum. Spindler (1934) concluded, from an experiment in which adult Nippostrongylus muris administered to rats by duodenal tube were found to inhibit egg production after later percutaneous infection, that the immunity against the parasite was localized in the intestine. This point of view was supported also by Chandler (1935e) from his work with Nippostrongylus muris in rats. He found, for example, that the transfer of the serum of immune rats to normal animals did not protect the latter against the parasite, even when the transfer was made from one to the other of parabiotic twins. He inferred that the immunity is largely local, a property of the intestinal mucosa, and that it may be nutritional in nature, antienzymes from the intestinal mucosa counteracting the enzymes by which the parasite digests the host tissue. In a consequent contribution he (1936h) agreed that generalized immunity to Nippostrongylus muris may follow transplantation of the parasite to the body cavity, since the number of larvae which mature following subsequent infection and their egg yield are reduced, but he maintained that a local intestinal immunity may develop which is not shared by the blood. Recently (1937) he has again expressed the belief that the immunity against parasites in the lumen of the intestine is local, stating that antigenic materials from the parasites often are not absorbed

but eliminated with the feces or other wastes. He reasoned that if these antibody-inciting substances are unable to reach the blood and thus do not stimulate the body as a whole, the immunity known to occur in such infections must result from the stimulation of tissue locally—e. g., epithelial cells at the sites occupied by the parasites. Sarles and Taliaferro expressed the belief that the immunity to Nippostrongylus muris is essentially localized in strategically placed organs—the skin, the lungs and the intestine—but that the antibody of the general circulation is the agent primarily responsible for the immunity (Taliaferro and Sarles, 1937a).

SPECIFICITY OF THE IMMUNITY

Relatively little work has been reported on the specificity of the immunity demonstrated with different helminths. Miller (1932e) was able to immunize rats against the cestode Cysticercus fasciolaris by introducing pieces of adult Taenia pisiformis, although no such resistance was produced by the administration of powdered Taenia pisiformis material. Partial protection of rats against Cysticercus fasciolaris followed oral or intraperitoneal introduction of materials from Hymenolepis sp. or Taenia saginata, although none at all followed the use of material from Diphyllobothrium latum or Dipylidium caninum (Miller, 1935j). Wigand found that a natural infection of Taenia pisiformis in the dog inhibited infection with Diphyllobothrium latum. The latter parasite developed to maturity and produced eggs but rapidly died out. This author pointed out that two helminths seldom inhabit the same part of the intestine simultaneously. Cross dissected 92 ciscoes from the Great Lakes region, finding only adult Proteocephalus and Acanthocephala of the genus Neoicanthorhynchus. When 15 or more Acanthocephala or 25 or more tapeworms were present, few or none of the alternate form were found, although when the total number of parasites was less, the two kinds were about evenly divided. The author believed that the results indicated a nonspecific immunity which limits either form when the other is present in large number.

Chandler (1932a) noted that the small amount of immunity that develops in rats against Nippostrongylus muris following injection of dead or live larvae is specific, since it does not result when larvae of even closely related forms are substituted as the immunizing agent.

(To be concluded)

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Walter V. Brem, at one time professor of pathology at the University of California, died in Los Angeles November 18, at the age of 62 years.

Einar Leifson has been appointed professor of bacteriology at the University of South Dakota.

John C. Bugher has resigned as assistant professor at the University of Michigan to join the staff of the Rockefeller Institute for Medical Research.

Richard H. Jaffé, pathologist at the Cook County Hospital, Chicago, died suddenly Dec. 17, 1937, at the age of 49 years.

Harry A. Davis, instructor in pathology at George Washington University, is serving as professor of pathology at the University of Tennessee in the place of Walter W. Brandes, who is on leave of absence because of illness.

Onie O. Williams has been appointed assistant professor of pathology at the University of Oklahoma.

Russell H. Holman has been appointed assistant professor of pathology at the University of North Carolina.

John A. Ferguson has been appointed assistant professor of pathology at the University of Vermont.

In addition to being chief of the division of pharmacology in the National Institute of Health, Dr. Carl Voegtlin has been appointed director of cancer research in the United States Public Health Service.

George H. F. Nuttall, professor of biology at Cambridge University died Dec. 16, 1937, at the age of 75 years. He was born in San Francisco and graduated in medicine at the University of California in 1884. His life was devoted to investigation and teaching in bacteriology, parasitology and hygiene. In 1904 he published the results of his well known pioneer work on blood immunity and blood relationship. He was founder and editor of the *Journal of Hygiene*.

The American Type Culture Collection.—This collection has been transferred from the John McCormick Institute for Infectious Diseases, Chicago, to Georgetown University School of Medicine, Washington, D. C. The curator is Dr. Mario Mollari.

Rackham Arthritis Research.—At the University of Michigan an extensive research in arthritis has been organized by means of grants from the Rackham Fund. Richard H. Fryberg will be the director of the research.

Fellowships for Cancer Research.—The Finney-Howell Research Foundation was created by the will of the late Dr. George Walker, Baltimore, to support research into the cause and treatment of cancer. Fellowships with an annual stipend of $2,000 will be awarded each year on the second Wednesday of March, beginning in 1938. The awards will be for one year with the possibility of renewal up to three years. Special grants of limited sums may be made to support the work carried on under a fellowship. Applications must be on file on or before February 1 at the office of the secretary of the foundation, Dr. William A. Fisher, 1211 Cathedral Street, Baltimore.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

The Vomiting Sickness of Jamaica, B. W. I. and Its Relation to Akee Poisoning. E. O. Jordan and W. Burrows, Am. J. Hyg. **25**:520, 1937.

The epidemiologic evidence available indicates that some of the cases of vomiting sickness continually occurring in Jamaica are akee poisoning. The seeds and pods of both ripe and unripe akees contain a substance which on ingestion induces violent vomiting in young cats and monkeys (Macacus rhesus). The arilli and placentae do not contain this substance. The distribution and properties of the akee poison substantiate and in no way contradict the epidemiologic evidence. The experimental evidence suggests that the akee poison may be a glucoside.

From the Authors' Summary.

Peptic Ulcer Produced by the Low Protein Diet. A. A. Weech and B. H. Paige, Am. J. Path. **13**:249, 1937.

Peptic ulcers of the stomach and duodenum developed in dogs maintained on a diet deficient in protein. Among twenty-two animals that received the diet for an average period of ninety days there were eight, or 36 per cent, that exhibited true peptic ulcers at autopsy. Of the remaining fourteen there were five, or 23 per cent, that showed erosions of gastric or duodenal epithelium without true ulceration.

From the Authors' Summary.

Specificity of the Thyrotropic Action of the Anterior Pituitary Gland. K. Emerson Jr., Bull. Johns Hopkins Hosp. **60**:358, 1937.

Extracts of beef ovary, purified by Loeser's method, produce no changes in the thyroid of the guinea-pig, whereas similar extracts of beef anterior pituitary cause good stimulation of the thyroid. The same holds true for the crude acetone-desiccated powder made from these two glands. Guinea-pig anterior pituitary powder produces definite stimulation in the guinea-pig thyroid if given in sufficiently large amounts or over a long enough period of time. The absolute concentration of thyrotropic substance is definitely less in guinea-pig than in beef anterior pituitary.

From the Author's Summary.

Antidiuretic Pituitary Substance in Blood. K. I. Melville, J. Exper. Med. **65**:415. 1937.

A method is described for quantitative extraction of posterior pituitary antidiuretic substance from blood with which it has been mixed in vitro and in vivo for experimental purposes. With this procedure, it is found that a similarly extractable active substance may be detected as a normal constituent of dog and human blood. The data obtained on the blood of normal pregnant women and on that of several pregnant women with early toxemia do not indicate any causal relationship between the presence of this substance in the circulating blood and the early symptoms (hypertension, edema, albuminuria) of the toxemia of pregnancy.

From the Author's Summary.

A Deficiency State Related to Protein Depletion. S. C. Madden, P. M. Winslow, J. W. Howland and G. H. Whipple, J. Exper. Med. **65**:431, 1937.

When the proteins in the blood plasma are depleted by bleeding, with return of washed red cells (plasmapheresis), a state is brought about in which the plasma protein in the circulation is steadily low and the production of plasma protein on a basal diet uniform. In such dogs the effects of various factors on the regeneration of plasma protein can be measured. Dogs previously the subjects of plasmapheresis appear during long rest periods to increase the stores of plasma protein building materials and the concentration of protein in the blood plasma above former normal levels. Such depletion of the plasma and intake of a basal diet over long periods (from twenty-five to thirty weeks) result in removal of much protein from the body fluids and tissues. At the same time the dog loses its appetite and may vomit some food, and shows loss of hair, a tendency toward ulceration of the skin and a distinct lowering of resistance to infection. The plasma protein may fall to fasting levels in spite of a food intake sufficient to maintain weight. It is believed that this condition represents a state of severe depletion of the essential protein matrix of the body cells.

From the Authors' Summary.

Experimental Nephritis in Rats Induced by Injection of Antikidney Serum. J. E. Smadel and others, J. Exper. Med. **65**:527, 541 and 557, 1937.

The glomerulonephritis induced in rats by so-called nephrotoxin was characterized clinically during its initial phase by severe albuminuria, cylindruria and anasarca but not by hematuria. Rapidly fatal nephritis was produced by injecting relatively large amounts of the antikidney serum at frequent intervals. In such cases the blood urea mounted rapidly, urea clearance fell, and death occurred within about two weeks. Milder nephritis of the chronic type was induced by giving smaller quantities of the antikidney serum in either single or divided doses. In these instances there was no immediate alteration of urea clearance. Lipemia and a deficit in plasma protein appeared with the development of anasarca. The majority of rats which survived the intial stage of this experimental nephritis continued to show marked albuminuria with casts until they died or were put to death months later. Some of these animals showed retardation of growth and a progressive fall in urea clearance. Terminally there developed marked retention of urea, a deficit in plasma protein, anemia and hypertension.

Administration of the relatively organ-specific antibody nephrotoxin, present in antikidney serum, is followed by diffuse glomerulonephritis. This is characterized early by swelling of the intercapillary substance of the glomerular tuft and by tubular degeneration. Fibrin thrombi are present in the glomerular capillaries only after the injection of antikidney serum results in a severe anaphylactoid reaction and are due to factors other than nephrotoxin. The urinary abnormalities which develop in every rat that has received a suitable injection of nephrotoxin usually continue until the animal dies or is put to death. The microscopic renal lesions of the early phase merge into scarring of the glomeruli and tubules. Histologic study of animals which die from three to eleven months after treatment reveals chronic progressive glomerulonephritis with generalized vascular lesions.

A saline extract of perfused rat kidney administered intravenously to a rat immediately before an injection of the antikidney serum by the same route prevents renal damage. A preliminary injection of physiologic solution of sodium chloride or of an extract of perfused rat liver has no preventive effect. These findings are a further indication that the nephrotoxic effect induced by antikidney serum is dependent on a relatively organ-specific antibody, nephrotoxin.

From the Authors' Summary.

PHYSIOLOGY OF THE HUMAN CERVICAL MUCOSA. A. WOLLNER, Surg., Gynec. & Obst. **64**:758, 1937.

Specimens of cervical mucosa were obtained for biopsy at weekly intervals from twenty women whose ages ranged between 26 and 39 years. A careful study of this material indicated that the belief that the cervical lining does not take part in the cytologic changes of the menstrual cycle is erroneous. Most characteristic are the variations taking place in the epithelium. As in the endometrium, so in the cervical mucosa destruction occurs at the onset of menstruation, following which, after the interval period, rapid regeneration and proliferation take place, although such a cleancut cycle cannot be demonstrated in many cases. The histologic picture in the premenstrual phase may resemble an inflammatory reaction, and cysts may result from exfoliation of glandular epithelium. Contrary to the statements that the cervical mucosa does not contain glycogen, Wollner demonstrated this substance not only in the epithelium but also in the glandular lumens. Owing to the ease with which specimens of mucosa can be obtained from selected areas, biopsy of this tissue may come to be the method of choice for studying the mechanism of the ovarian cycle.

WARREN C. HUNTER.

BIOLOGIC SIGNIFICANCE OF LYMPH NODES IN RELATION TO TRIBUTARY TERRITORY IN NORMAL AND IN PATHOLOGIC CONDITIONS. G. MOTTURA, Arch. per le sc. med. **63**:169, 1937.

Mottura states that the lymph nodes, because of their structure and their relation to the connective tissue and blood capillaries, are concerned with the purification of lymph from catabolic products and proteins, as well as with parenteral digestion and reabsorption of the products. In the course of great reabsorption and inflammation, either clinically or experimentally induced, an intense reaction of the regional lymph nodes takes place. The morphologic aspect of the nodes shows processes of antiacid protection and decomposition of the proteins. The alterations indicate local reactions of humoral protection and parenteral digestion. The reaction takes place by mobilization of reticulo-endothelial cells, lymphatic hyperplasia and certain physicochemical changes of the cells. The lymph nodes are extremely reactive to catabolic products and proteins in the lymph. The intensity of the reaction depends on the condition, physiologic or pathologic, of the tributary organ. The lymph nodes react to pathologic conditions by an intense reaction that causes plugging of the sinuses, by exudation and local inflammation, with consequent temporary stoppage of their functions in the lymph circulation.

Pathologic Anatomy

VASCULAR COMPLICATIONS OF POLYCYTHEMIA. I. L. NORMAN and E. V. ALLEN, Am. Heart J. **13**:257, 1937.

Study of ninety-eight cases of polycythemia vera and thirty-five cases of relative polycythemia reveals that erythromelalgia, myocardial infarction, angina pectoris, occlusive disease of the peripheral arteries, cerebral hemorrhage of thrombosis, intra-abdominal vascular thrombosis, phlebitis and vasomotor neurosis occur in about a third of the cases. Recognition of the relationships is important diagnostically and therapeutically.

FROM THE AUTHORS' SUMMARY.

SIGNIFICANCE OF BLOOD VESSELS IN HUMAN HEART VALVES. L. GROSS, Am. Heart J. **13**:275, 1937.

Described in this report are the findings in 100 nonvascularized normal human valves, 44 human hearts in which the valves were vascularized but appeared grossly normal, 13 hearts with extinct monovalvular rheumatic disease, 50 other human hearts, 50 calf hearts, and a number of rabbit and guinea-pig hearts which had been given injections by Wearn's technic, as well as a number of calf, swine,

rabbit and guinea-pig hearts which had not received such injections, and swine, ox and human embryos, serially sectioned. The so-called normal vascularized human hearts presented widespread stigmas which in incidence and distribution bore a striking resemblance to the findings in specimens that presented undisputed extinct rheumatic inflammation. Reasons are given which indicate very strongly that rheumatic fever which has gone on to complete healing is responsible for the formation of these blood vessels. It is further shown that rheumatic fever can produce muscular vessels as one of the results of the evolution of granulation tissue. A description is given of the normally vascularized calf heart valves, and attention is drawn to the differences between these valves with their blood vessels and those sometimes found in human hearts. It is further shown that while ox and swine embryos display blood vessels in their valves these are not found in the heart valves of human embryos. As a consequence, there exists no embryogenetic basis on which to explain the occurrence of the blood vessels found in human hearts. A discussion of injection technic, together with new observations, reenforces the belief that such technic affords no information on the problem under discussion which cannot be obtained better by microscopic observations on serial sections. The conclusion is drawn that blood vessels do not exist in normal valves or, if they do, that they must be very rare.

FROM THE AUTHOR'S SUMMARY.

A STUDY OF THE OSSEOUS REMAINS OF THE MOUND BUILDERS OF EASTERN ARKANSAS. E. G. WAKEFIELD, S. C. DELLINGER and J. D. CAMP, Am. J. M. Sc. **193**:488, 1937.

As a rule, the skeletal remains of the long extinct Mound Builders of eastern Arkansas were well formed and well proportioned, which indicates that they had an excellent muscular system. Anterior bowing or lateral deformities of the bones of the legs, such as are seen in rickets, were not found. Irregular epiphysial lines were not seen in any of the roentgenograms. The legs were straight, the pelves narrow and the arms of moderate length. The heads would have been well formed if they had not been subjected to certain self-inflicted deforming procedures during their growth. In such cases the anteroposterior diameters were decreased and the bregmatic and lateral diameters were increased; these changes produced flat heads. In some of these deformed heads there were symmetrical depressions above the orbits. One can be certain that the shape of these heads was not the result of disease but the result of cradling or molding during growth. Examples of the so-called "infectious arthritis" were not found. Hypertrophic changes about the margins of the knee joints and vertebrae were occasionally present. There were no changes characteritic of tuberculosis. An excellent example of spondylitis deformans was present in three thoracic vertebrae. Osteomyelitis was present to extreme degrees in several specimens. The teeth were usually good. Healed tooth sockets indicated antemortem extractions of teeth. Cauterizing or scraping of diseased long bones of the legs was a fairly common procedure. Fractured long bones were set with fair precision. Gross and roentgenologic evidences of the bony changes which may be seen in certain anemias were observed in some bones. One can be reasonably sure that when these lesions are present in the cranium and are accompanied by changes in the long bones the person concerned had a blood dyscrasia. The diagnosis of syphilis among precolonial Americans who lived in Eastern Arkansas cannot be made absolutely. It is the authors' opinion that sufficient evidence is present to say that they had it. It is important to emphasize that the tibias and other long bones which were studied and which are described in this report were well preserved. The authors are entirely in accord with the findings of Williams and Means, namely, that syphilis was endemic in North America before known contacts with Europeans. However, the findings recorded here are not presented as evidence of either the new or the old world origin of syphilis.

FROM THE AUTHORS' SUMMARY.

FIBROSIS OF THE BONE MARROW WITH A LEUKEMOID BLOOD PICTURE. S. R. METTIER and G. Y. RUSK, Am. J. Path. **13**:377, 1937.

Two cases of fibrosis of the bone marrow associated with a leukemoid blood picture are presented. In the first case the onset of illness was characterized by leukopenia and a hemorrhagic tendency, and later, with moderate enlargement of the spleen and terminal leukocytosis. In the second case the course of illness for a period of three years prior to the patient's death was typical of leukemia. In both cases during the postmortem examination there were found in the liver, spleen and lymph nodes changes compatible with a diagnosis of leukemia, but there was partial obliteration of the marrow cavities with fibrous tissue and bony spicules.

FROM THE AUTHORS' SUMMARY.

THE AORTIC COMMISSURAL LESION IN RHEUMATIC FEVER. L. GROSS and G. SILVERMAN, Am. J. Path. **13**:389, 1937.

There have been described in this report the findings in seventy cases of rheumatic fever, segregated into six groups according to the clinical course taken by the disease. It is shown that a number of inflammatory changes are found in the aortic root, wedge, annulus, ring, subaortic angle and pericardial mantle which are characteristic of rheumatic fever and which to some extent reflect the clinical course of the disease. Even when healing takes place, the histologic characteristics of the commissural lesion afford additional stigmas which are of value in discovering a past rheumatic process. A discussion of the pathogenesis of this lesion is given, from which it appears that even though the original infection may reach the aortic ring through several routes, in most instances the inflammatory granulation tissue passes from the pericardial mantle through the aortic root, wedge and annulus to reach the aortic ring. The latter shows a much more flagrant inflammatory process which spreads into the valve leaflets and, probably with the additional factor of trauma caused by the systolic and diastolic movements of the cusps, eventually leads to their agglutination. The possible significance of these findings in relation to the pathogenesis of the so-called congenital bicuspid aortic valve is indicated. A description is also given of the histologic and topographic changes taking place during the different age periods in the normal aortic commissural region.

FROM THE AUTHORS' SUMMARY.

ANATOMIC FEATURES OF THE CARDIAC ORIFICE OF THE STOMACH, WITH REFERENCE TO CARDIOSPASM. F. C. LENDRUM, Arch. Int. Med. **59**:474, 1937.

The results are compatible with the theory of Hurst, namely, that cardiospasm, or achalasia of the cardia, is primarily a disease of the myenteric plexus. Hurst expressed the opinion that with the destruction of the myenteric plexus the sympathetic fibers to the terminal portion of the esophagus are allowed to act unopposed. As a result, the terminal portion of the esophagus does not relax normally with the swallowing reflex, and dysphagia results.

FROM THE AUTHOR'S SUMMARY.

INTESTINAL EPITHELIUM IN THE GASTRIC MUCOSA. H. A. MAGNUS, J. Path. & Bact. **44**:389, 1937.

The gastric mucosa has been examined to determine the frequency of occurrence and the distribution of intestinal epithelium in the stomach. It has been shown that this epithelium is identical in every way with true intestinal epithelium. Evidence supports the view that intestinal epithelium in the stomach is the result of faulty regeneration of surface epithelium in a mucosa repeatedly damaged by gastritis and that the finding is, in fact, an example of metaplasia resulting from chronic irritation. Emphasis is laid on the necessity of examining large strips of gastric mucosa.

FROM THE AUTHOR'S SUMMARY.

THE WEIGHT OF THE PARATHYROID GLANDS. J. R. GILMOUR and W. J. MARTIN, J. Path. & Bact. **44**:431, 1937.

The weights of the parathyroid glands of 527 patients were studied. These patients were grouped as (1) normal, (2) having endocrine abnormalities, (3) showing abnormalities of the bones, (4) having toxic nephritis and (5) having other renal diseases. The histologically normal glands showed the average weight in the adult male to be 263 mg. for the glands and 174 mg. for the parenchyma; that in the adult female, 295 mg. for the glands and 193 mg. for the parenchyma. The weight of the glands as a whole ranged from 3 mg. in an infant 6 hours old to 388 mg. in a man of 45 years. The maximal weight was attained in males between 21 and 30 years and in females at 50 years. The mean percentage volume showed a slight decline with age. The two lower glands were heavier than the two upper, and the order of increasing weight was right upper, left upper, right lower and left lower. The parathyroid and parenchyma were of greater weight in the adult female than in the adult male of the group with endocrine abnormalities, and the parathyroid but not the parenchyma was of greater weight in the female of this group than in the female of the normal group. The groups with renal diseases showed histologic evidence of abnormal activity.

FROM THE AUTHORS' SUMMARY.

VARIATIONS IN THE RETICULOCYTES OF RABBITS. P. NICOLLE, Arch. Inst. Pasteur de Tunis **26**:68, 1937.

An exhaustive study of rabbits during gestation and thereafter indicated that marked reticulosis occurred as the end of the period of gestation approached, followed by a sharp drop before birth occurred. A second rise was noted during lactation, less marked and more immediate if suckling was not permitted. On the other hand, reticulosis was very marked (over 8 per cent) following the removal of the young after some days of suckling. The possible functional role of reticulocytes in the formation of milk and the possible dependence of their titer on hormones active in gestation and in lactation are considered.

M. S. MARSHALL.

SEROUS HEPATITIS IN MAN. K. HEINEMANN, Beitr. z. path. Anat. u. z. allg. Path. **99**:1, 1937.

Although the fully developed serous hepatitis of Rössle is extremely rare, "pericapillary edema," with separation of the epithelial cells from the reticular framework, and "capillary mobilization" of doubtful origin are common. The changes about the capillaries, provoked by chronic congestion, consist of pericapillary edema, thickening and numerical increase of the reticular fibers, and mobilization of the endothelial cells. These changes accord with those described by Rössle in serous hepatitis. A relationship of pericapillary edema to definite diseases was not successfully established. However, it occurs with a certain regularity in chronic congestion of cardiac origin, in bile stasis and in septic processes.

R. J. LEBOWICH.

SYPHILITIC MYELOSIS. M. STAEMMLER, Beitr. z. path. Anat. u. z. allg. Path. **99**:34, 1937.

Staemmler points out that syphilitic myelosis has received little attention in the textbooks of pathology. In a study of eight spinal cords, it was observed that the process was commonly most severe in the thoracic region, attacking first the subpial tracts and later spreading into the posterior columns and nerve roots. In the more severe process, the lateral columns were involved, and at times the anterior columns. The gray matter was spared. The degeneration was diffuse and never systematic in distribution. The leptomeninges were almost regularly the seat of a chronic productive inflammation of variable intensity. Histologically it concerned a purely degenerative change, characterized by a marginal subpial

zone of gliosis, a middle zone of loosened, vacuolated white matter containing many fat granule cells and the remains of intact myelin sheaths, and an inner normal zone situated immediately about the gray matter. The author assumes that spirochetes or products of their degeneration passing from the spinal fluid into the cord are responsible for the lesions. Depending on the localization and intensity of the process, the clinical picture may be that of tabes or of pyramidal tract disease.

R. J. LEBOWICH.

PATHOGENESIS OF CHOLANGITIS. LA MANNA, Virchows Arch. f. path. Anat. **298**: 44, 1936.

For his purpose La Manna defines cholangitis as an inflammatory reaction of the excretory biliary system from the bile capillaries to the papilla of Vater. When the gallbladder is also involved, he uses the term "total cholangitis." Bacteriologic and experimental investigations have failed to clear up the pathogenesis of the condition under investigation. Intelligent interpretation of pathologic-histologic observations is still a serviceable investigative procedure. In his study La Manna selected from more than 300 necropsies in Rössle's institute cases of generalized infection without jaundice, cases of jaundice without generalized infection and cases of jaundice with generalized infection. Each group contained cases with and without cholangitis, as determined by histologic investigation. For the latter purpose material was taken from various portions of the liver and of the extrahepatic biliary system. Almost invariably when cholangitis was observed the process was older in the distal or extrahepatic portion of the system than in the intrahepatic portion. Involvement of the adjacent liver by direct continuity from an inflamed gallbladder was rarely observed. Excretion of bacteria into the bile is admitted and is accepted as almost physiologic. But this phenomenon is not evidence of inflammatory involvement of the biliary system, since cholangiolitis without older involvement of the lower lying portions of the system was so rarely seen. La Manna concludes that in the vast majority of instances cholangitis is an ascending infection and is only occasionally hematogenous. Concomitant chronic inflammation of the appendix was observed in about a third of the cases of chronic cholecystitis. But La Manna denies the occurrence of a condition to which the term "cholecysto-appendicitis" has been applied. He holds that there is no evidence that cholecystitis is secondary to or dependent on chronic appendicitis. While the association of the two processes is admitted, they are not dependent on each other in their pathogenesis.

O. T. SCHULTZ.

WERNICKE'S DISEASE IN NONALCOHOLIC PATIENTS WITH CANCER. C. NEUBÜRGER, Virchows Arch. f. path. Anat. **298**:68, 1936.

Gamper is credited by Neubürger with being the first to observe that in Wernicke's disease with the Korsakoff syndrome in severe chronic alcoholism the chief pathologic change is located in the mamillary body. Later investigators described similar changes in other severe exogenous intoxications, and in 1932 and 1934 Neubürger reported two cases of carcinoma with similar pathologic changes in the mamillary body. He now summarizes fourteen instances of the characteristic pathologic alterations in nonalcoholic patients. Ten of them had malignant disease, seven having carcinoma of the stomach. His concept is that toxic aromatic derivatives of intermediary metabolism in the presence of a damaged liver have a selective affinity for the mamillary body and the adjacent tissues of the brain. These substances act primarily on the mesenchymal tissues. The blood vessels are injured, leading to hemorrhage, which is often evident macroscopically. Mesenchymal and glial proliferation follow.

O. T. SCHULTZ.

GOLGI APPARATUS AND ASH OF MULTINUCLEAR GIANT CELLS. H. OKKELS, Acta path. et microbiol. Scandinav. **13:** 383, 1936.

In mesenchymatous cells the Golgi apparatus varies greatly in shape and size. In fibroblasts it is ill defined and small. In plasma cells, polyblasts and epithelioid cells it is distinctly contracted as a rule and located near the nucleus. In multinuclear cells it is less coherent, and in giant cells it may undergo complete disintegration. The mineral content of giant cells as determined by incineration varies, being more or less abundant according to the nature of the disease in question. Foreign body giant cells may be rich in inorganic material, especially those in silicotic lesions.

Pathologic Chemistry and Physics

A NEW GLYCOLYSIS ACTIVATOR. W. FABISCH, Am. J. Cancer **28:**764, 1936.

The method of hot methyl alcohol extraction (suggested by M. Ascoli and Izar's technic of preparing antigen for the miostagmin reaction) enabled Fabisch to obtain from the blood corpuscles and serum of tumor-bearing human beings, as well as from Jensen sarcoma and the organs of sarcomatous rats, substances causing an average 75 per cent increase of aerobic glycolysis in normal human blood corpuscles. Corresponding extracts from normal human blood and normal rat organs caused an increase of only about 20 per cent. The working hypothesis that the available substances are identical with the substances activating glycolysis could not be experimentally confirmed. Availability of these substances and increase in glycolysis are to be regarded not as cause and effect but rather as concomitant results of a higher cause. Concerning the nature of the substances activating glycolysis it can be said only that they probably belong to the group of fats and lipoids and that the double unions which they include could hardly determine their efficiency.

FROM THE AUTHOR'S SUMMARY.

STUDIES OF GALL BLADDER FUNCTION: XIV. J. JOHNSON, A. L. ELLIS and C. REIGEL, Am. J. M. Sc. **193:**483, 1937.

A method has been devised to compare the absorption of sodium tetra-iodophenolphthalein from the normal gallbladder of the dog with that from the damaged gallbladder. From 83 to 100 per cent of the dye was recovered from the normal gallbladder, and from 5.8 to 68 per cent from the damaged gallbladder, after twenty-four hours. The variation depended at least in part on the degree of damage. In other words, sodium tetra-iodophenolphthalein is absorbed more rapidly from the damaged than from the normal gallbladder. It is reasonable to suppose that this factor may play some part in the failure to make the diseased gallbladder visible in clinical cholecystography. Sodium tetra-iodophenolphthalein is not excreted by the liver in the bile in the same form as it is given intravenously. The data obtained regarding the rate of absorption of the dye by the normal gallbladder further strengthen Johnson's contention that the rapid changes which occur during clinical cholecystography must be explained on the basis that the dye is expelled through the cystic duct rather than absorbed by the gallbladder.

FROM THE AUTHORS' SUMMARY.

PHOSPHORUS CONTENT OF THE BLOOD SERUM DURING EPILEPTIC SEIZURE. A. WEIL and E. LIEBERT, Arch. Neurol. & Psychiat. **37:** 584, 1937.

Weil and Liebert found that during, before or following epileptic seizure the phosphorus (inorganic acid soluble and not acid soluble) in the blood serum is increased by 34 per cent. This is true not only in man but also in experimental

animals. The cause of the increase was found in the muscular contractions, for when in experimental convulsions these contractions are checked by curare or in man are replaced by equivalents, the content of phosphorus remains normal.

G. B. HASSIN.

CHEMICAL ANALYSIS OF BLOOD AND CEREBROSPINAL FLUID (IN SCHIZOPHRENIA). S. KATZENELBOGEN, Arch. Neurol. & Psychiat. **37:** 881, 1937.

Katzenelbogen carried out biochemical studies on the spinal fluid and blood of twenty schizophrenic patients. The patients had been subject to psychosis for from three to fifteen years. Altogether, nineteen chemical constituents were determined, and in only three patients were they found to be within normal limits. In the remaining patients, some components were found to be abnormal, either in the blood or in the spinal fluid or in both. The most frequent deviations from the normal were in the potassium and lactic acid contents of the blood and of the spinal fluid; the next most frequent were in the cholesterol content of the spinal fluid, the globulin content of blood, and the calcium, total protein and globulin content of the spinal fluid.

G. B. HASSIN.

CENTRIFUGATION STUDIES. W. J. ELFORD and C. H. ANDREWES, Brit. J. Exper. Path. **17:** 399 and 422, 1936.

With the ordinary bucket type of high speed centrifuge, the sedimentation of bacteria, bacteriophages, viruses and proteins was studied. When a capillary tube was inverted into the main bulk of liquid contained in the centrifuge bucket, sedimentation occurred in the inverted cell. This arrangement minimized mixing and simplified the process of sampling, since the cell on being withdrawn from the liquid retained the sample to be tested. The size of particles in any suspension was calculated from measurements of the average concentration within the cell at the beginning and end of a period of controlled centrifugation by use of a relationship based on Stoke's law. By this method the following densities were found: Bacillus prodigiosus, 1.10; Staphylococcus bacteriophage (Elford and Andrewes, 1932), 1.25; vaccinia virus, 1.18, and influenza virus, 1.20. The sizes of the particles were found to be as follows: B. prodigiosus from 0.7 to 0.8 micron; Staphylococcus K phage, from 60 to 70 millimicrons, S 13 phage (antisalmonella; Elford and Andrewes, 1932), from 15 to 17 millimicrons; hematocyanin (Helix), 22 millimicrons; edestin, 8 millimicrons; vaccinia virus, from 170 to 180 millimicrons; influenza virus, from 87 to 99 millimicrons, and the virus of Rous sarcoma, from 60 to 70 millimicrons. These figures corresponded with those found in ultrafiltration studies.

FROM THE AUTHORS' SUMMARY. (FREDERICK STENN.)

HISTOCHEMICAL DETECTION OF LEAD IN BONE. F. TIMM, Virchows Arch. f. path. Anat. **297:** 502, 1936.

A method is described for the histochemical detection of lead in bone. It reveals that lead is normally present in bone. The essentials of this method are precipitation of the lead by means of hydrogen sulfide and examination of frozen decalcified sections under dark field illumination. Copper and tin, which are also normally present in bone and are precipitated by hydrogen sulfide, may be removed by the proper reagents, which do not affect the lead sulfide.

O. T. SCHULTZ.

GLUTATHIONEMIA IN TUBERCULOSIS OF BONES AND JOINTS. M. M. ALTSCHULER, Acta tuberc. Scandinav. **10:** 370, 1936.

In tuberculosis of the bones and joints in children the glutathione in the blood increases. The fluctuations in the glutathione correspond to the intensity of the tuberculous process.

Microbiology and Parasitology

RELATIONSHIP BETWEEN YAWS AND SYPHILIS. T. B. TURNER, Am. J. Hyg. **25**:477, 1937.

A study of yaws among persons living in rural Jamaica and of syphilis among persons living in Baltimore showed easily recognizable differences between the two diseases. These differences cannot be explained on the basis of differences in such factors as race, age at the time of infection, portal of entry of the organism or social and economic status. Certain climatic factors seemed to play a role in determining the distribution of yaws, but there was no clear evidence that these factors materially modified the course of either yaws or syphilis in the victim. Typical cases of each disease were found among persons living in the same environment in Kingston, Jamaica. The inoculation of rabbits with treponemes from cases of each disease produced lesions which differed according to the disease with which the patient was suffering. Each of thirteen strains of the spirochetes of yaws gave rise to lesions of the same type in rabbits. Each of eight strains of the spirochetes of syphilis recovered from persons living in Jamaica produced lesions which, while similar to each other, were readily distinguishable from those produced by the spirochetes of yaws. These observations confirm those made previously on strains of spirochetes isolated from persons with yaws in Haiti and strains of spirochetes recovered from persons with syphilis living in the United States, Europe and Haiti. The ability to produce characteristic lesions in the rabbit was not lost on serial passage in this animal over a period of three years. It is concluded, first, that Spirochaeta pallida possesses pathogenic properties which differ from those of Treponema pertenue and, second, that the differences noted between yaws and syphilis in man are due, in part at least, to inherent differences between the causative agents of these diseases.

FROM THE AUTHOR'S SUMMARY.

PATHOGENESIS OF TYPHOID FEVER. E. W. GOODPASTURE, Am. J. Path. **13**:175, 1937.

Early in cases of typhoid fever small gram-negative intracellular bacilli, judged to be Eberthella typhi, have been found at autopsy in the cytoplasm of young plasma cells, otherwise apparently unaltered, located in the lymphoid follicles of iliac and mesenteric lesions. Larger gram-negative bacilli have been found in macrophages in the intestinal lesions in association with remains of phagocytosed lymphocytes or necrotic remnants of the macrophages themselves. It is concluded that E. typhi is capable of growing in both these situations and under the conditions indicated. The interpretation is proposed that the young plasma cell is an essential host for E. typhi in the typical human disease and serves as a nourishing and protecting medium not only during the period of incubation but throughout the active course of the disease.

FROM THE AUTHOR'S SUMMARY.

EXPERIMENTAL PULMONARY TUBERCULOSIS IN THE RABBIT. E. M. MEDLAR and K. T. SASANO, Am. Rev. Tuberc. **34**:456, 1936.

From data obtained in the study of tuberculous lesions in rabbits under various experimental conditions it is concluded that allergy is not the prime reason why localizing tuberculous lesions proceed to cavitation; that alteration of the tissue caused by a primary infection is not the prime reason why in reinfection localized tuberculous lesions cavitate, as similar lesions were obtained from a first infection; that localizing cavitating lesions require a certain balance between virulence and dosage, as well as resistance of the host plus localizing of bacilli in certain areas of lung parenchyma; that higher portions of the lung appear to be less resistant to infection; that the site of progressing tuberculous lesions can be changed in the rabbit through posture. It is suggested that the so-called adult type of pulmonary tuberculosis is due in large part to the lodging of the bacilli in the less resistant upper units of the lung. No definite reason for the greater susceptibility of certain portions of the lung can be given.

H. J. CORPER.

ANTHRACOSILICOSIS AND TUBERCULOSIS. M. J. SOKOLOFF, Am. Rev. Tuberc. **34**:700, 1936.

Recent statistics indicate that tuberculosis occurs more frequently in coal miners than was formerly believed. This increase is concomitant with changed working conditions in coal mines, which expose the workers not only to carbon particles but also to a tremendous amount of fine particles of silica. To determine the incidence of tuberculosis as a complication of anthracosilicosis a group of 418 coal miners were studied while institutionalized because of chronic disabling pulmonary disease. Tuberculosis was found in more than half (56.7 per cent) of this group; in the majority of these patients (92 per cent) it occurred after the age of 30 years and in association with pneumonoconiosis in the third stage (70.1 per cent). Tuberculosis associated with anthracosilicosis is distinctive, consisting of a wide scattering of caseous tuberculous nodules throughout the lungs rather than the usual apical distribution of uncomplicated tuberculosis. There are two types: In the nontoxic type, symptoms of anthracosilicosis predominate, while in the other the toxemia of tuberculosis is the distinguishing feature.

H. J. CORPER.

PATHOGENESIS OF ERYTHEMA NODOSUM, WITH SPECIAL REFERENCE TO TUBERCULOSIS, STREPTOCOCCIC INFECTION AND RHEUMATIC FEVER. W. W. SPINK, Arch. Int. Med. **59**:65, 1937.

Ten patients with erythema nodosum were critically studied. Evidence of tuberculosis was present in only one patient. In five of the patients erythema nodosum was preceded by a sore throat, and cultures of the throats of four revealed Streptococcus haemolyticus of the beta type. Intradermal injection of a streptococcus endotoxin (nucleoprotein) produced nodules similar to the lesions of erythema nodosum in eight of the ten patients; the streptococcic nodules and the lesions of erythema nodosum revealed the same histologic appearance; similar lesions were produced by injection of broth filtrates of streptococci isolated from two of the patients. The same picture has been produced by injection of tuberculin. An analysis of the records of 133 cases of erythema nodosum at the Boston City Hospital from 1924 to 1934 revealed a similar causal relationship to streptococcic infections and, in addition, to rheumatic fever. Erythema nodosum appears to be a nonspecific inflammatory reaction of the skin to a variety of bacterial, toxic and chemical agents.

FROM THE AUTHOR'S SUMMARY.

EPIDEMIC ENCEPHALITIS (ST. LOUIS TYPE) IN TOLEDO, OHIO. K. LÖWENBERG and T. ZBINDEN, Arch. Neurol. & Psychiat. **36**:1155, 1936.

On the basis of clinical and pathologic observations in two of the fifty cases of encephalitis that occurred in Toledo, Ohio, in the summer of 1934, the authors concluded that the Toledo epidemic was similar to those observed in Japan and St. Louis. The type of encephalitis represented, they think, should be termed encephalitis B, in distinction from Economo's type, known as epidemic or lethargic, which should be classified as encephalitis A. The word epidemic should be omitted, as both types may be epidemic. Clinically encephalitis B is more severe, has a more rapid course and gives a higher percentage of mortality. The histologic changes are more diffuse. They affect the cortex and subcortex alike, with profuse formation of "microglial" nodules and cuffs of perivascular infiltrations and marked involvement not only of the midbrain but also of the diencephalon, pons (both basilar and dorsal parts) and cerebellum. The hypothalamus and meninges are only mildly involved. Badly involved is the gray substance of the spinal cord, especially the anterior horns, but changes are also present in the white substance.

G. B. HASSIN.

THE CAPSULE OF PASTEURELLA SEPTICA. F. W. PRIESTLEY, Brit. J. Exper. Path. **17**:374, 1936.

A capsular envelop similar to that described for Pasteurella pestis is shown to be present around the virulent but not around the avirulent form of Pasteurella septica. This capsule appears after incubation for twenty-four hours at 37 C. Incubation at a higher or a lower temperature tends to inhibit its formation. Icubation for more than twenty-four hours results in loss of capsular substance. The medium used has little or no effect on this. The capsule is very labile to heat, being destroyed at 56 C. in from forty-five to sixty minutes and almost instantaneously at 100 C. Antigenically, virulent organisms are more complex than avirulent ones. In both there is a common somatic antigen, but the virulent organism has, in addition, an antigen associated with the capsular envelop.

FROM THE AUTHOR'S SUMMARY.

FIÈVRE BOUTONNEUSE AND TICK-BITE FEVER. A. PIJPER, Arch. Inst. Pasteur de Tunis **25**:388, 1936.

Virus of Fièvre *boutonneuse* secured from Tunis did not in tests give cross-immunity to virus of tick fever from eastern South Africa. It is concluded that these diseases are not identical.

TRANSMISSION OF TYPHUS VIRUS FROM SICK TO HEALTHY LICE. A. V. PCHENICHNOF and B. I. RAIKHER, Arch. Inst. Pasteur de Tunis **25**:402, 1936.

Typhus virus may be transmitted from sick lice to healthy lice. Healthy lice, on biting the skin of a person infected by crushing lice with typhus, can be infected with the virus by way of the mouth. There exists in lice an intestinal infection which provokes, by way of the buccal cavity, a mass epizootic with a characteristic period of incubation and symptoms. The virus of the louse epizootic is easily passed by mouth from louse to louse. Infection by mouth derived from a meal on infected skin permits the preservation of the virus in the same way as in an epizootic of lice. Passage in lice by way of the mouth does not submit the virus to any appreciable modification. The possibility of prolonged conservation of the virus in lice marks a new stage in the study of the conservation of the virus during interepidemic periods.

FROM THE AUTHORS' CONCLUSIONS.

CULTIVATION OF TUBERCLE BACILLI FROM THE BLOOD AND ORGANS AFTER DEATH. H. POPPER, A. J. LESER and L. GERZNER, Virchows Arch. f. path. Anat. **297**:368, 1936.

In confirmation of previous work it was possible to cultivate tubercle bacilli from the blood at necropsy by the Löwenstein method in 70 per cent of 100 persons who died of tuberculosis. Positive cultures were obtained in a much higher percentage after death than during life. This is held to indicate that in tuberculosis there is a terminal massive bacteremia, which may be the actual cause of death. It was also possible in a high percentage of the cases to cultivate the organism from organs which revealed no gross or microscopic evidence of tuberculosis. Such positive cultures are believed to have been derived from the blood contained in the organs.

O. T. SCHULTZ.

Immunology

ESSENTIAL IMMUNIZING ANTIGEN OF THE TYPHOID BACILLUS. L. D. FELTON and F. B. WAKEMAN, Bull. Johns Hopkins Hosp. **60**:178, 1937.

An essential immunizing fraction of the typhoid bacillus has been isolated which chemically is a polysaccharide. Immunologically it is more active than the total amount of the acetone-extracted dried typhoid bacilli from which it is derived.

FROM THE AUTHORS' SUMMARY.

A Heat-Stable, Serologically Active Substance on Tissues Infected with Vaccine Virus. R. F. Parker and T. M. Rivers, J. Exper. Med. **65**:243, 1937.

A method has been described by which a stable serologically active substance has been isolated in a relatively pure state from tissue infected with vaccine virus. It has the characteristics of an alcohol-soluble protein and is not precipitated by boiling in a neutral aqueous solution. In a dilution of 1:640,000 it gives a precipitate when mixed with a serum containing antibodies against Craigie's S antigen of vaccine virus, but no visible reaction occurs when it is mixed with serum depleted of S antibodies by means of absorption.

From the Authors' Summary.

Antibody Response of Human Subjects to the Virus of Human Influenza. T. Francis Jr. and T. P. Magill, J. Exper. Med. **65**:251, 1937.

Human influenza virus cultivated in tissue culture medium may be administered subcutaneously or intradermally to human beings without giving rise to evidence of infection. Subjects so treated show a good titer of circulating antibody effective against mouse passage virus. If antibody was previously present, vaccination stimulates the production of more of it. The antibody so induced persists for at least five months, although during this time a decline in titer may have begun. The antibody response to vaccination parallels in extent and persistence that to the naturally acquired disease. The available data do not enable one to evaluate the effect of vaccination in the prevention of human influenza. It seems not unlikely that the increase in circulating antibody is accompanied by an increase in ability to combat the natural infection. From the Authors' Summary.

Shwartzman Phenomenon in Lymph Nodes. L. H. Koplik, J. Exper. Med. **65**:287, 1937.

Characteristic changes are produced in the lymph nodes of rabbits by injecting intravenously certain bacterial filtrates twenty-four hours after intralymphatic or intradermal injection of the same filtrates. These changes are limited to the nodes served by the lymphatics into which the filtrate was injected or to those furnishing lymphatic drainage for the sites of intradermal injection. By either method the filtrate of the initial, or preparatory, injection reaches the lymph nodes through one or more of the latter's afferent lymphatics, and similar lesions are produced in the nodes with both methods. The lesions consist of hemorrhages, recognizable by gross and microscopic examination. The capillaries and veins are congested and thrombosed. Their endothelial cells are swollen. Arterioles are generally little affected. Though hemorrhages and thromboses are usually seen together in the nodes, they have been observed occurring independently. They are probably secondary to endothelial changes. The lesions are not dependent on the amount of preexisting inflammation in the nodes. Endothelial changes, hemorrhages and thromboses are usually noted in the regional nodes when positive Shwartzman reactions have been elicited in prepared skin by intravenous injection of the bacterial filtrate. However, in many instances these lesions were observed under similar conditions in these nodes even when the Shwartzman reaction in the skin was negative. It appears that lymph nodes are more susceptible to the production of the Shwartzman phenomenon than the cutaneous sites which they drain. A single intralymphatic or intradermal injection of any bacterial filtrate used in this study, even if this is in high concentration, does not produce in adjacent lymph nodes the characteristic changes noted when this preparatory injection is followed by a subsequent intravenous injection of the filtrate. A single intravenous injection also is not productive of hemorrhage and thrombosis in lymph nodes.

From the Author's Conclusions.

MENINGOCOCCUS PRECIPITINOGENS IN THE CEREBROSPINAL FLUID. H. E. ALEXANDER and G. RAKE, J. Exper. Med. **65**:317, 1937.

In cases of meningococcic and other forms of meningitis precipitin tests have been carried out on the spinal fluids with monovalent antimeningococcus horse serum of high titer. It has been possible thus within two hours to diagnose and type type I and type II meningococcic meningitis. In a certain number of cases fluids which revealed no precipitation when first drawn gave positive evidence of the disease and its type after standing for one or two days at 37 C. or at room temperature. In 9.5 per cent of all type I cases the reactions of the fluids did not become positive. Fluids in cases due to atypical meningococci may react with the type serum of the group to which they belong (i. e., types II and VII, which belong to group II, react with type II serum) but do not in every case. In cases of meningitis not due to meningococci the fluids give no reaction. The use of concentrated serum is not advantageous at present owing to the heterologous reactions which occur.

FROM THE AUTHORS' SUMMARY.

PURIFICATION OF THE ACTIVE PRINCIPLE OF LOCAL SKIN REACTIVITY TO BACTERIAL FILTRATES. G. SHWARTZMAN, S. MORELL and H. SOBOTKA, J. Exper. Med. **65**:323, 1937.

A quantitative biologic assay of products obtained by dialysis of filtrates from culture of B. typhosus, the meningococcus and B. coli was undertaken. It was found that the active principle of the phenomenon of local skin reactivity to the bacterial filtrate was retained by the cellophane membrane. An appreciable purification was thus affected, represented in about a threefold increase in reacting potency per milligram of dry weight and of nitrogen, on the average. Attempts to purify bacterial filtrates by fractional precipitation of their concentrates with dilute alkali, acid and alcohol were unsuccessful.

FROM THE AUTHORS' SUMMARY.

ANAPHYLACTIC SENSITIZATION WITH CHEMICALLY DEFINITE COMPOUNDS. H. E. FIERZ, W. JADASSOHN and W. STOLL, J. Exper. Med. **65**:339, 1937.

Sodium atoxyl-diazo-amino-sulfo-anthranilate injected into guinea-pigs produces anaphylactic hypersensitiveness to the corresponding azoprotein (Schultz-Dale test). This leads to the conclusion that the injected sodium atoxyl-diazo-amino-sulfo-anthranilate first decomposes and then couples with the body protein to form the corresponding azoprotein and that it is this compound which sensitizes.

FROM THE AUTHORS' SUMMARY.

MOLECULAR WEIGHT OF ANTIBODIES. M. HEIDELBERGER and K. O. PEDERSEN, J. Exper. Med. **65**:393, 1937.

Highly purified rabbit antibody to the carbohydrate of Pneumococcus type III proved to be homogeneous in the ultracentrifuge, and its sedimentation constant, 7.0×10^{-13}, did not differ from that of the principal component of normal rabbit globulin or of immune rabbit globulin containing up to 50 per cent of antiegg albumin. The molecular weight of the antibody in the rabbit is therefore probably very close to that of the principal component of normal globulin, namely, 150,000. Highly purified horse antibody to the carbohydrate of Pneumococcus type I, on the other hand, was homogeneous in the ultracentrifuge only when prepared from serum stored without preservative. Its sedimentation constant, 18.4×10^{-13}, coincided with that of the principal component of globulin in most of the Felton solutions and purified antibody solutions studied. The molecular weight of the horse antibody to pneumococcus carbohydrate is probably from three to four times that of the principal component of normal globulin. The significance of the differences between the antibody formed in the rabbit and that in the horse is

discussed. The results are given of ultracentrifuge studies on the molecular species in solutions of egg albumin-antiegg albumin specific precipitates dissolved in excess egg albumin. The implications of the results are discussed.

FROM THE AUTHORS' SUMMARY.

INFLUENZA: ACTIVE IMMUNIZATION OF MICE. C. H. ANDREWES and W. SMITH, Brit. J. Exper. Path. **18**:43, 1937.

Subcutaneous and intraperitoneal inoculation of living influenza virus into mice confers on them substantial immunity against intranasal infection. Two doses confer much better immunity than one. Influenza virus can be partially purified by washing on a collodion membrane, without great loss of titer; this washed virus immunizes mice as effectively as does crude virus. Influenza virus inactivated by a 1:5,000 or a 1:10,000 dilution of formaldehyde immunizes mice almost or quite as effectively as does living virus. Virus inactivated by formaldehyde as strong as 1:1,000 still makes a good vaccine. Formaldehydized vaccine keeps its potency, at least in part, for two months in the cold. Influenza virus which has been both purified by washing and inactivated by formaldehyde makes a relatively poor vaccine for mice. Preliminary experiments on a few human beings indicate that subcutaneous inoculation of influenza virus inactivated with formaldehyde will produce in at least some subjects a substantial rise in their neutralizing antibody for influenza virus. The W. S. strain of influenza virus appears in the course of passages through mice to have undergone a rather sudden spontaneous change of such a nature that it is now possible to obtain filtrates of from ten to one hundred times as high a titer as formerly.

FROM THE AUTHORS' SUMMARY.

ERYTHROGENIC STREPTOCOCCUS (DICK) TOXIN IN PUERPERAL INFECTION. M. KENNY and L. COLEBROOK, J. Path. & Bact. **44**:91, 1937.

Skin tests with Dick toxin in 100 early cases of puerperal infection (all the patients infected by hemolytic streptococci) were positive in 19 instances, i. e., in a rather smaller percentage than among normal women at or about full term. Contrary to experience in scarlet fever, none of the positive reactors gave a negative reaction when retested after the lapse of from six to thirty-seven days. In two fatal cases of puerperal peritonitis in women who had presented an intense scarlatiniform rash during life the filtered peritoneal exudate was found to contain an erythrogenic toxin of considerable potency when tested on human beings, and this toxin was neutralized by standard antitoxin. One of the filtrates on intradermal injection caused a sharp febrile reaction.

FROM THE AUTHORS' SUMMARY.

VACCINATION AGAINST YELLOW FEVER. A. W. SELLARDS and J. LAIGRET, Arch. Inst. Pasteur de Tunis **25**:424, 1936.

Various arguments, as well as tests on animals, were used to evaluate the efficacy of a vaccine prepared from living virus of yellow fever modified by intracerebral passage in mice. With some 20,000 vaccinations on human volunteers, some of these were certainly exposed to infection, and no cases occurred. On a volunteer it was reasonably demonstrated that he had not had yellow fever, that following vaccination his serum became protective and that seven months later, on submitting to being bitten by infected mosquitoes, there was no illness whatever. The bites of these infected mosquitoes killed two control monkeys. The authors grant that the record will not remain perfect but claim sufficient evidence is now at hand to warrant general vaccination if an epidemic occurs.

M. S. MARSHALL.

SEROLOGY OF MALARIA. G. VILLAIN and R. DUPOUX, Arch. Inst. Pasteur de Tunis **25**:469, 1936.

An attempt was made to provide a basis for an explanation of the melanin flocculation test for malaria. The possible antigens in malaria include both specific and nonspecific products. The specificity of the melanin reaction probably does not rest on true immunizing antibodies, however. Practically, the authors' reaction with artificial melanin appeared to give a reaction diagnostically as valuable as Henry's reaction. FROM THE AUTHORS' CONCLUSIONS.

Medicolegal Pathology

QUANTITATIVE DETERMINATION OF THE ALCOHOL CONTENT OF BLOOD. F. SCHWARTZ, Schweiz. med. Wchnschr. **67**:54, 1937.

In quantitative estimation of the alcohol content of the blood in relation to motor vehicle accidents, Schwartz' method of determining the degree of alcoholism may prove helpful. The results of urinalysis are unreliable. An exact method is afforded by analysis of the brain for alcohol after death. These conclusions were reached after study of over 1,000 cases. If the value of the alcohol in the blood is less than 0.5 per cent, the effect of the alcohol cannot be significant, since with this concentration most persons show no signs of acute alcoholism. When the concentration in the blood is between 0.5 and 1 per cent, most persons are free from alcoholic symptoms, though women and young persons with concentrations near 1 per cent may show symptoms of alcoholism. These values are not a sufficient basis for legal action. Concentrations between 1 and 1.5 per cent are found in persons showing signs of alcoholic intoxication, such as excitement and paralytic manifestations. Excitation caused by alcohol of this concentration results in traffic accidents and disregard of rules for safety. Paralysis, excitation and impairment of consciousness occur when the concentration is between 1.5 and 2 per cent, though in certain persons symptoms may not be present with concentrations as high as 2.3 per cent. Concentrations of 2 per cent or more indicate complete inability to cope with traffic problems. A sample of blood should be obtained promptly after the accident. FREDERICK STENN.

A METHOD FOR DETERMINING THE TIME OF DEATH. E. KARLMARK, Deutsche Ztschr. f. d. ges. gerichtl. Med. **27**:326, 1937.

The claims for the method of determining the time of death are: 1. It may be employed when several days have elapsed between the time of death and the necropsy. 2. It is not dependent on the outer appearance of the body and therefore may be used on the bodies of persons who have been burned. 3. The medical examiner does not have to be present at the first examination by the police. 4. A statement can be made as to what time in the twenty-four hours of a day the person died provided that accurate knowledge concerning the habits of the deceased person as regards eating and sleeping are known.

Sources of error exist if illness interfered with the normal functions of the person whose death is examined, or if there were great differences in the intake of food, e. g., eating late at night, or if there was marked variation in the hydrochloric acid of the gastric juice.

The method rests on the regular movements of the gastro-intestinal tract. Food ordinarily leaves the stomach in from four to six hours and therefore is mixed with bile from the liver and gallbladder. Since the liver excretes more bile than can be stored in the gallbladder, there is a passage of bile into the duodenum about every one and a half hours, which is carried along in the bowel by peristalsis. The periods of bile flow can be determined if clamps are applied to the bowel to prevent admixture of the bile with the mucus which has been moved along in the bowel between the gushes of bile. By counting the alternating bile-containing and bile-empty zones in the bowel proximal to the last meal the time of death can be determined.

The author also makes reference to a series of tables which he has compiled from a study of the effects of digestion on a variety of foods for stated periods.

GEORGE J. RUKSTINAT.

DIFFERENTIATION OF MURDER AND SUICIDE BY HANGING. W. NEUGEBAUER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **28**:111, 1937.

Deaths apparently due to hanging are classified by Neugebauer as suicidal, simulated suicide and murder. In suicides there is invariably a deep furrow on the neck produced by the suspending cord. This occurs even when a cloth has been first placed about the neck by a meticulous person before applying the noose. A variety of superficial injuries may be found on the bodies of persons who thresh about in their death agonies. Menstrual blood soiling furniture or carpets may lead to erroneous assumptions of genital injuries. Self-inflicted genital injury followed by hanging is cited as an occasional problem. An illustrative case is that of a woman who thrust a long knife between the left labia into the pelvis, injuring the anterior wall of the bladder. Without withdrawing the knife, she made a further thrust, which injured the back wall of the bladder, severed the mesentery and entered the front edge of the liver. To make sure of death, she had previously placed a noose about her neck and suspended the rope from the door frame.

Frequently the victim of murder by strangulation is found with a rope about the neck which has been placed there in order to divert suspicion from murder to suicide. In most such instances, however, diligent search will disclose thumb marks and nail scratches on the neck of the victim. Most important is the lack of tension on the suspending rope and the absence of a rope furrow on the neck. Typical hemorrhages in the conjunctivae, neck muscles, and skin also serve to establish that the death was due to strangulation.

Murder by hanging is infrequent and occurs only if the murderer is much stronger than his victim or if a group of people participate in the hanging. Evidences of the forcible suspension of the body are usually found on the rope in the form of superficial scuffing from the friction of the rafter or the branch of the tree.

GEORGE J. RUKSTINAT.

COMPARISON OF THE O SUBSTANCE IN NEW-BORN INFANTS AND IN ADULTS. B. ELMENHOFF-NIELSEN, Ztschr. f. Rassenphysiol. **8**:81, 1936.

By means of titration and absorption tests with the α_2 agglutinins present in certain ox serums the author found that the O receptor in group O new-born infants has reached a fourth of the usual strength. On the other hand, the O substance was found to be present in larger amounts in new-born infants of groups A and AB than in adults of groups A and AB. This was true especially in those new-born infants in whom the A receptor was least developed. By immunizing a rabbit possessing preformed α_2 agglutinin with group O blood, an immune anti-O serum was obtained, though not of a very high titer (approximately 1:256).

A. S. WIENER.

FORENSIC DETERMINATION OF TYPES OF STAINS. F. THERKELSEN, Ztschr. f. Rassenphysiol. **8**:98, 1936.

This paper deals with the determination of the blood groups and of the M-N types of blood and seminal stains by the absorption method. With regard to the demonstration of the A-B groups, fresh blood has a greater absorbing capacity than dry blood stains, which in turn absorb better than extracts. On the other hand, the absorbing capacities of fresh seminal fluid, seminal stains and seminal extracts are approximately the same, and many times greater than the absorbing capacity of blood in equivalent amounts taken from the same persons who supplied the semen. The demonstration of the A agglutinogen in A_2B blood stains could be effected only by using special anti-A serum. With regard to the

properties M and N, these are present in the blood cells but not in the semen. Property M can readily be demonstrated by the absorption technic in fresh and dry blood; however, certain testing fluids do not give reliable results and should not be used for this purpose. On the other hand, consistent results were not obtained when an attempt was made to demonstrate property N by the absorption technic. For forensic purposes, therefore, when the absorption technic is used on blood stains, only tests for M, not those for N, are trustworthy. There is no change in the absorbing capacity of stains kept for half a year either in daylight or in the dark, in the icebox, at room temperature or at 37C. There was slight diminution in the absorbing capacity when the stains were kept at 56 C. Direct exposure to sunlight for forty hours and irradiation by means of a quartz lamp had no effect.

A. S. WIENER.

Technical

A COMPARATIVE STUDY OF THE MCNEIL AND WITEBSKY ANTIGENS USED IN THE SERODIAGNOSIS OF GONORRHEA. A. HOLLANDER, Am. J. Syph., Gonor. & Ven. Dis. **21**:140, 1937.

The Witebsky and McNeil antigens were used in testing 507 gonorrheal serums and 484 nongonorrheal serums, the methods used being interchanged with each antigen. The results show that both antigens are almost exactly equally sensitive and specific. They also prove that with a good antigen the technic may be varied and a sufficiently high percentage of positive results still obtained to be of value to the physician.

FROM THE AUTHOR'S SUMMARY.

GONADOTROPIC HORMONE IN THE BLOOD AND URINE OF EARLY PREGNANCY. H. M. EVANS, C. L. KOHLS and D. H. WONDER, J. A. M. A. **108**:287, 1937.

Charts are presented showing the actual quantitative content of the urine in gonadotropic hormone at various times throughout six normal pregnancies. The charts show the invariable existence of an exceedingly steep and high hormone peak at a time which is quite accurately one month from the beginning of the first expected but missed menstruation. The peak must be recognized as a normal phenomenon in all studies attempting to relate high levels of hormone with pathologic insignia.

FROM THE AUTHORS' SUMMARY.

SEROLOGIC DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS. ISRAEL DAVIDSOHN, J. A. M. A. **108**:289, 1937.

The test for heterophilic antibodies is of confirmatory value in the diagnosis of infectious mononucleosis with typical clinical and hematologic features.

It is decisive (*a*) for the early recognition of this disease when it shows unusual hematologic and clinical signs, some of which are due to complicating factors, and (*b*) for the exclusion of conditions that are otherwise clinically and hematologically indistinguishable from infectious mononucleosis.

The differential test for infectious mononucleosis is decisive (*a*) for the exclusion of conditions that are clinically and hematologically indistinguishable from infectious mononucleosis and that have a so-called borderline titer of heterophilic antibodies (1:56 or 1:112); (*b*) for the recognition of infectious mononucleosis in a late stage, when the titer of heterophilic antibodies is relatively low, and (*c*) for the recognition of the disease when it is complicated by a recent therapeutic injection of immune horse serum or by serum disease.

FROM THE AUTHOR'S SUMMARY.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

FRANCIS D. GUNN, *President*

Regular Monthly Meeting, Nov. 8, 1937

EDWIN F. HIRSCH, *Secretary*

PATHOGENESIS OF AMEBIC ABSCESSES OF THE LIVER AND A FURTHER STUDY OF CHANGES IN THE LIVER IN AMEBIASIS. REX B. PALMER.

The material was obtained at the Cook County Hospital, Alexian Brothers Hospital and Evanston Hospital during the past seven years. The patients ranged in age from 31 to 72 years; fifteen were white males, two were Negroes, and one was a white female. Peritonitis complicated the amebic dysentery in seven; syphilis, in two; carcinoma with metastasis in the liver, lymphosarcoma and vegetative endocarditis, in one each. Thirteen of the nineteen patients had abscesses of the liver; five had multiple abscesses. In three patients the capsules of the abscesses were less than 3 mm. thick; in the others the walls varied from 4 mm. to 2 cm. The diagnosis of amebic dysentery was made clinically in only six cases. The duration of symptoms varied from ten days to three years, the average period being slightly more than two months. The liver was enlarged in twelve patients; in four it was not, and in two it was small. The portal connective tissue in eighteen patients was increased markedly or moderately and irregularly. The marked fibrous tissue reaction in nine patients was associated with proliferation of the bile ducts. An increase of the round cells in the portal triads was observed in ten. Fatty changes of the hepatic lobules occurred in nine and was most marked midway between the central vein and the portal triads. Pigmentation of the liver cells or of the Kupffer cells occurred in ten. The increase of fibrous tissue was greatest about the large abscesses. At the margins of large abscesses, islands of liver tissue were surrounded by fibrous thickenings of the portal triads. The liver tissues were compressed and atrophic near the large abscesses, and the cords of liver cells tended to be parallel to the capsules of the abscesses. Most of the abscesses were deep in the right lobe of the liver. The multiple abscesses in one liver had thin walls, and there was no increase of the periportal connective tissue. The duration of the symptoms in this patient was ten days. The patient was 72 years of age and had bilateral bronchopneumonia and diffuse peritonitis. The duration of the amebic disease was too short for the usual changes to occur. Amebas were found in a thrombosed branch of the portal vein in the liver of this patient.

Amebas gain entrance to the liver by the portal vein, and abscesses originate in the portal triads. Others have observed amebas invading capillaries near the intestinal lesions. Amebas were seen in a thrombosed portal vein in one of the livers studied. Abscesses began below the capsule of the liver in 84 per cent of the cases in this series. Such abscesses begin with lysis of the liver parenchyma about the portal triads, associated with only a slight inflammatory reaction. They spread irregularly into the surrounding tissues and increase in size by the coalescence of neighboring abscesses. Tissues of the portal triads are more resistant than the surrounding liver parenchyma and can be recognized in the large abscesses. The centrally liquefied regions, which occasionally contain recognizable liver cells, have a thin fibrous margin with a moderate number of lymphocytes and large mononuclear cells. Polymorphonuclear leukocytes are found rarely. The hepatic sinusoids about the small abscesses are usually moderately engorged with blood cells. An increase of portal connective tissue is important in the walling-off of large abscesses. It occurs also with active intestinal lesions not associated with

abscesses of the liver. Definite hepatitis occurs with active amebic lesions in the colon. This was indicated by the increase of the portal connective tissue in all but one of the livers studied and by the parenchymatous degeneration, fatty changes, round cell infiltration, hemosiderosis and lysis of hepatic cells.

POTENTIAL DANGERS OF INTRANASAL MEDICATION. PAUL R. CANNON and THEODORE E. WALSH.

Intranasal instillation into healthy rabbits of small amounts of several commonly used therapeutic agents dissolved in light mineral oil led to rapid development of acute pulmonary edema and lipoid pneumonia. So-called mild antiseptic agents, such as "mild silver salts," and astringents, such as trinitrophenol, zinc sulfate, tannic acid and sodium aluminum sulfate, similarly led to acute pulmonary edema, necrosis and bronchopneumonia. Vasoconstricting agents and mild antiseptic agents dissolved in aqueous or saline solutions caused little, if any, damage to pulmonary tissues after intranasal instillation.

The improper use of oily solutions and of irritating and toxic substances in the noses of children with acute infections of the upper respiratory tract is potentially hazardous, because the simultaneous aspiration of bacteria and chemicals into the lungs may favor bacterial growth in devitalized and edematous tissue and cause serious or fatal bronchopneumonia.

DISCUSSION

F. D. GUNN: Is there any explanation for the increased permeability of the capillaries peripherally in the lungs and the resulting pulmonary edema as contrasted with the central portions without edema?

S. R. ROSENTHAL: Were the animals under anesthesia during the experiment?

PAUL R. CANNON: The dilution of the irritant seems to be the only important factor in the distribution of the edema fluid. Some alveoli contained fibrin; many, simply a transudate. The rabbits were not anesthetized during the experiments and were in their normal postures. In this posture the oil gravitates into the upper and intermediate lobes rather than into the lower lobes of the lungs.

METASTASIZING FIBROMYOMA OF THE PLEURA. R. H. JAFFÉ.†

Two large tumors of the pleura composed of smooth muscle fibers and connective tissue are described. One originated from the parietal pleura covering the right half of the diaphragm and showed extensive calcification and focal ossification. It caused the patient's death by obliterating the intrapericardial portion of the inferior vena cava, thus producing enormous congestion of the abdominal viscera. The other tumor protruded into the pleural cavity from the right posterior wall of the thorax. It contained xanthomatous tissues and was observed incidentally in a woman who had died from an advanced carcinoma of the cervix uteri. Although both tumors were histologically benign, tumor tissue of identical structure was in the lymph nodes that drained them. The lesions in the lymph nodes are considered to be metastases of the pleural tumors. As in other viscera, fibromyoma of the pleura may produce distant metastases.

SARCOMAS PRODUCED BY BENZPYRINE AND METHYLCHOLANTHRENE. ALEXANDER BRUNSCHWIG.

Primary tumors of bone have been produced experimentally by irradiation. Daels and Sabin, Forkner and Doan produced fibrosarcoma of bone by injecting mesothorium. Ludin produced chondrosarcoma by roentgen radiation in one rabbit. Schürch and Uehlinger produced ossifying sarcoma by roentgen radiation in several rabbits. So far as could be determined no previous reports have been made of primary tumors of bone produced by carcinogenic compounds. In each of fifty mice a small pellet of 5 per cent benzpyrine in cholesterol was inserted into the

† Dr. Jaffé died Dec. 17, 1937.

shaft of the tibia by performing an arthrotomy of the knee joint and making an opening in the articular surface of the tibia. Three animals survived for from eight to nine months. One had a firm fusiform mass in the upper portion of the tibia, roentgenograms of which were typical of an ossifying sarcoma. Three weeks after the first manifestations of the tumor, the animal became acutely ill and died. Postmortem examination demonstrated abscesses of the liver and lungs. Portions of the tumor in this mouse were transplanted into twelve other mice. Within seventy-two hours all of these died of an acute disease associated with multiple abscesses of the lungs and liver. The mouse with the tumor obviously had had a septicemia of some highly virulent organism, and the other animals were infected by transplantation of the tumor tissue. Microscopic examination of the tumor showed a typical ossifying sarcoma.

Similar experiments with crystals of methylcholanthrene were made in a series of twenty-five rats. In some cases the crystals were packed into a small hole made in the shaft of the tibia, and in others 5 per cent methylcholanthrene-cholesterol pellets were injected into the lower part of the shaft of the femur through an arthrotomy of the knee joint. This group has been followed over a year. To date one animal has shown development of a tumor. This tumor appeared twelve months after injection of the methylcholanthrene-cholesterol pellet and consisted of a dense mass arising from and completely surrounding the lower third of the femur. On palpation it did not contain bone, and this impression was confirmed by roentgenograms. The tumor grew rapidly, infiltrating the muscles of the thigh, and produced finally a mass about the size of a hen's egg. The animal was killed, and the peripheral portions of the tumor were cut away. A roentgenogram showed disintegration of the lower epiphysial region and rather marked osteolysis of the lower femoral metaphysis, giving an appearance similar to fibrosarcoma of bone in man. Microscopic examination showed the tumor to consist of large and small spindle cells, many in mitosis. It filled the lower portion of the medullary cavity and extended toward the trochanteric region. A limited amount of reactive bone of periosteal origin was also observed over portions of the shaft included in the tumor. There was no evidence of true bone tumor.

An ossifying sarcoma (malignant osteoblastoma) of the tibia and a fibrosarcoma of the femur were produced in a mouse and in a rat, respectively. The former developed eight months after implantation of a 5 per cent benzpyrine-cholesterol pellet, and the latter twelve months after implantation of a 5 per cent methylcholanthrene-cholesterol pellet.

DISCUSSION

E. F. HIRSCH: Was cartilage found in the bone tissues and do you think there is any possibility that the bone lesion could have been a parosteal callus?

R. H. JAFFÉ: Did the benzpyrine change the composition of the blood?

A. BRUNSCHWIG: There was no cartilage in the bone tissues. The development of the bone lesion is unlike that of a parosteal callus. Studies of the blood were not made. In another group of about twenty-five mice receiving benzpyrine, lymphatic leukemia developed in one mouse.

FRANCIS D. GUNN, *President*

Regular Monthly Meeting, Dec. 13, 1937

EDWIN F. HIRSCH, *Secretary*

VITAMIN C AND RESISTANCE OF THE GUINEA PIG TO INFECTION WITH BACTERIUM NECROPHORUM. NORMAN B. MCCULLOUGH.

The relation of a deficiency of vitamin C in the guinea pig to infection with various strains of Bacterium necrophorum was studied. Avirulent human strains that did not produce infection in guinea pigs on a diet adequate in vitamin C pro-

duced minor abscesses in the extremely scorbutic guinea pigs. Animals with subacute scurvy did not show infection. Vitamin C therapy resulted in prompt recovery of infected scorbutic animals. The slightly more virulent animal strains, which produced transient abscesses in the control animals, gave a more severe degree of infection in the guinea pigs deficient in vitamin C. The lesions persisted and the guinea pigs died much sooner than did uninfected animals deficient in vitamin C. A severe scorbutus was necessary before a drop in resistance to infection with Bact. necrophorum became evident.

DISCUSSION

I. PILOT: The lesions about the teeth of patients with scurvy are associated with enormous numbers of fusospirochetal organisms. From the blood of such patients a nonhemolytic streptococcus was isolated but not the anaerobic bacteria. When fruit juices and fresh vegetables are given, the alveolar mucosa heals, and the anaerobes diminish in number and even disappear. These observations in patients agree with those in the experiments.

S. ROSENTHAL: In experimental tuberculosis excessive doses of vitamin C exert no influence, but if there is a deficiency in the vitamin the tuberculosis is more extensive.

P. R. CANNON: Were studies of the blood made in the vitamin-deficient animals, and were there changes in the bone marrow?

NORMAN B. MCCULLOUGH: The blood and bone marrow were not studied, but anemia does not occur in scorbutic guinea pigs. Polymorphonuclear leukocytes seemed less numerous in the lesions of animals with scurvy.

CONGENITAL ABSENCE OF ABDOMINAL MUSCLES. B. F. LICHTENSTEIN.

From an anatomic standpoint, congenital absence of individual muscles is not rare. Clinically, however, only those muscular defects are recognized which produce functional disturbances or changes in the contour of the body. Congenital absence of abdominal muscles has unusual interest because of the frequent coexistent disorders of the genito-urinary system. A boy aged 3 years had had since birth a greatly protuberant abdomen. On entrance to the Cook County Hospital, he had acute pharyngitis, and the urinary bladder was distended up to the umbilicus. There was bilateral cryptorchidism. Palpation of the abdomen after 1,400 cc. of urine had been removed by catheter revealed that the abdominal musculature was absent and that the internal viscera could be outlined easily. Cystoscopy revealed a greatly dilated and atonic urinary bladder, and pyelography demonstrated marked bilateral hydronephrosis and hydro-ureter. There was no evidence of obstruction to the urinary outflow. Neurologic examination revealed nothing abnormal other than absence of the abdominal reflexes.

Postmortem examination demonstrated acute generalized purulent peritonitis. Grossly there was no evidence of muscle in the anterior abdominal wall. The testicles were at the brim of the pelvis. The urinary bladder was greatly distended and the wall thickened. The ureters were tortuous and dilated, and there was bilateral hydronephrosis. Microscopic examination of the anterior abdominal wall showed complete absence of striated muscle. Sections through the lower thoracic levels of the spinal cord revealed diminution in the size and number of the large motor ganglion cells without reactive glial or vascular phenomena. The white substance was unchanged. The muscle defect was interpreted as primary aplasia and the ganglionic cell changes in the spinal cord as evidences of retrograde degeneration or disuse atrophy.

The kidneys in the later months of fetal development secrete urine into the amniotic fluid. The emptying of the urinary bladder is normally accomplished by contraction of its musculature, supplemented by an increase in the intra-abdominal pressure produced by contraction of the diaphragm and the abdominal muscles. The absence of the abdominal musculature in the fetus in utero precluded

the utilization of this supplemental force, and the urinary bladder was unable to empty its contents against the pressure of the amniotic fluid. This produced the dilatation of the urinary passages, later followed by hypertrophy of the wall of the urinary bladder.

DISCUSSION

EDWIN F. HIRSCH: Was the colon dilated?

B. F. LICHTENSTEIN: There were no changes.

EXPERIMENTAL STUDIES ON INTESTINAL OBSTRUCTION. SARAH B. HAEREM, G. M. DACK and L. R. DRAGSTEDT.

Of the various theories proposed to explain death in intestinal obstruction, two have received the most attention, namely: that it is due to the loss of large amounts of fluid from the upper part of the gastro-intestinal tract by vomiting, and that it is due to the absorption of some toxic product formed in the obstructed bowel. What role bacteria play and how permeable obstructed segments of bowel are to bacterial products are unsettled problems which have prompted this investigation.

Bacteriologic studies were made on fluid obtained from closed intestinal loops in eight dogs. Clostridium welchii and Bacterium coli were found to be the predominating organisms. In most instances, the isolated Welch organism was capable of producing a potent toxin in vitro, but no definite evidence of the presence of the toxin of this organism in the fluid of the loop was demonstrated.

The permeability of the obstructed bowel has been studied by other workers with regard to some of the chemical poisons, but no satisfactory method has been devised for studying the permeability to bacterial products. Since dogs are known to be resistant to Clostridium botulinum toxin and since this toxin is of bacterial origin, it was thought that these animals might be better for studying the conditions found in intestinal obstruction.

The toxin of Cl. botulinum does not pass through the normal intestinal walls of dogs when fed in quantities of 50 cc. or less. With larger quantities occasionally there is absorption, but never in appreciable amounts. This toxin injected intraperitoneally in suitable doses is readily absorbed into the blood stream. It may be demonstrated in the blood within two hours, increases in quantity with time and is lethal to the dog.

Absorption of the toxin of Cl. botulinum placed in small quanties in obstructed segments of the small intestine of the dog has been demonstrated. The distention of the isolated loops was followed in x-ray films, and some correlation seems to exist between the demonstration of toxin in the blood stream and the distention of the loops. Gross necrosis of the distended segments did not always occur when there was absorption, but devitalization of the bowel segments greatly facilitated the appearance of toxin in the blood stream.

DISCUSSION

P. R. CANNON: Were there any distinctive changes in the wall of the obstructed bowel, such as hyperemia or hemorrhage, associated with the absorption of the toxin?

S. ROSENTHAL: What was the nature of the diet? In experiments on appendicitis in dogs a protein diet was associated with necrosis, whereas a carbohydrate diet was not.

H. B. FISHBACK: Was any attempt made to determine whether the absorption was through the portal blood vessels or through the lymph channels?

SARAH B. HAEREM: Distention was the only significant change in the obstructed loop except in one with necrosis, from which absorption of toxin took place rapidly. The animals did not receive food during the experiment, and before it they had a regular diet. No attempt was made to determine a portal or a lymphatic absorption of toxin from the bowel.

A Study of Some Immunologic Factors in Chronic Staphylococcic Osteomyelitis. Katherine E. Hite, Sam Banks and G. M. Dack.

Treatment of chronic staphylococcic osteomyelitis with toxoid and antitoxin has been disappointing, although favorable results have been obtained with these agents in acute and generalized infections. In the present study consideration has been given to organisms isolated from lesions, antihemolysin in the patient's serum and the local inflammatory reaction as factors contributing to these unfavorable results. Study of the fermentation of mannitol and lactose, the liquefaction of gelatin and the production of coagulase by cultures revealed no essential difference between strains from osteomyelitis and those from other sources. Assay of the alpha and beta hemolysin and dermonecrotoxin produced by ninety-three strains, seventy-four of which were isolated from osteomyelitis, showed statistically that the strains from osteomyelitis produced somewhat less potent toxins. However, some strains yielded highly potent filtrates. The difference was not considered to be of sufficient magnitude to be a major factor in the immunology of the disease. Determinations of antihemolysin were made on numerous samples of serum collected over long periods of time and during various stages of the disease from twenty-one patients. In each patient the antihemolysin was found to rise during or immediately following periods of acute exacerbation and rapidly became reduced during the chronic stages. A similar relationship was reflected in the titers of patients from whom only single samples were obtained. Patients given toxoid during the chronic stages responded with a rapid rise in antihemolysin but showed no clinical change.

The results described suggest that the local tissue reaction occurring inhibits the exchange of materials between the lesion and the circulation. This possibility was tested both in patients and in experimental sinuses in the subcutaneous tissues of rabbits. Typhoid vaccine introduced into chronic sinuses of four patients resulted in no increase in agglutinins for Bacterium typhosum. In rabbits the production of agglutinins for Bact. typhosum and of precipitins against horse plasma was small when the corresponding antigens were introduced into the experimental tracts. Cl. botulinum antitoxin similarly injected failed to protect against intravenous homologous toxin in eleven rabbits. In one in vitro experiment the lack of general response was not due to the presence of the inflammatory exudate.

DISCUSSION

S. Rosenthal: Besredka contends that antiserum should be given at the site of infection. The beneficial effects from this are much greater than those when the antiserum is given intravenously. These observations emphasize the importance of Besredka's views.

NEW ENGLAND PATHOLOGICAL SOCIETY

Frederic Parker Jr., *President*

Regular Meeting, Nov. 18, 1937

J. B. Hazard, *Secretary*

Incidence of Nodules in Thyroids as Observed in Routine Necropsies in a Nongoitrous Region. S. L. Gargill, M.J. Schlesinger and I. H. Saxe.

A study was made of the relative frequency of nodular and non-nodular thyroids in autopsy material available in Boston. The material consisted of 2,185 protocols of recent complete autopsies in three large teaching hospitals. In only 1,373 of these autopsies had the thyroid been examined; in the others the gland had not been examined because of limitation of incisions or other restrictions. Since the

primary interest was in the clinical significance of nodules in the thyroid, the selection of the material was limited to nodules 1 cm. or more in diameter. A nodule of this size, it was felt, should be clinically palpable, especially if it were in an exposed position.

In this series of routine autopsies 8.2 per cent of adequately examined thyroids contained nodules which should have been clinically palpable. The incidence of such nodules was higher in women than in men by about 2 to 1. Such nodules were rare in both men and women under 30 years of age. In women over 50 years of age the average incidence was 15.2 per cent, and in a selected group it was close to 40 per cent. Most of these nodules were of the colloid nodular type, and the majority showed nonmalignant degenerative changes. Of the seventy-four nodular thyroids that were microscopically examined, 8.1 per cent proved to harbor malignant neoplasms.

In a man or a woman under the age of 30 in a nongoitrous district, a nodule in the thyroid should be considered potentially malignant and should be surgically extirpated. Between the ages of 30 and 50 years, because of the general higher susceptibility to carcinoma, this potentiality becomes greater. A larger proportion of such nodules, however, surgically investigated at this later age, will prove benign. In the thyroids of persons past the age of 50, nodules are so common as to be almost physiologic.

DISCUSSION

SHIELDS WARREN: There are in Dr. Gargill's paper a number of points of interest brought out in such a way as to be of value to all who are present here. One point that interested me particularly was that although among those showing the development of these nodules the females outnumbered the males 2 to 1, there is nevertheless considerable predominance of this type of thyroid disease in males as far as thyroid change in general is concerned. Instead of the 2 to 1 reported here, if one takes thyrotoxicosis, colloid goiter of a clinically evident type, or if one takes malignant growth, the ratio runs very strikingly about 7 to 1 in favor of females.

The second point of definite value is the emphasis on the increase in colloid with age. One of the characteristics of the aging thyroid is the deposition of more and more colloid, so that the development of colloid nodules is practically physiologic and of decided clinical significance, because a nodule of the colloid type in a person over 50 is quite less apt to be malignant than one in a person under 50.

B. E. CLARKE: What are the criteria of malignancy in an encapsulated nodule in the thyroid?

SAMUEL L. GARGILL: Clinically, we have no criteria to differentiate a benign from a malignant nodule. Dr. Schlesinger can answer that question better in regard to the pathologic picture.

M. S. SCHLESINGER: Five out and out carcinomas and one sarcoma were present in this group of thyroids, and there was no question as to the malignancy of the nodules. None of the six malignant tumors in this group fall into the class that comes under the head of blood vessel-invading adenoma.

HEMOLYSINS ASSOCIATED WITH ACUTE HEMOLYTIC ANEMIA. WILLIAM DAMESHEK and STEVEN O. SCHWARTZ.

Three cases of severe anemia were recently observed, characterized by an acute downhill course, splenomegaly and acholuric jaundice. In two the anemia was of the macrocytic variety, and the fragility test was normal. In the third there was microspherocytosis, and the fragility test showed a grossly abnormal index (from 0.72 to 0.44). The administration of liver extract and transfusions of blood were of no value, but splenectomy performed as an emergency measure was followed by prompt and dramatic recovery. Tests of the serums for hemolytic activity demonstrated the presence of an unusual hemolytic factor in all three cases; i. e., there was shown: ability to cause hemolysis of red cells of all the blood groups; evidence of autohemolysis; inactivation of hemolysis by heating

the serum to 56 C.; reestablishment of activity on the addition of guinea pig complement; gradual diminution in hemolytic activity with prolonged standing at icebox temperature; suppression of hemolytic activity on incubation at 37.5 C. with normal human serum before the red cell suspension was added; an individual variability in the hemolytic susceptibility of cells from different subjects of the same blood group.

The microscopic picture of the spleen differed in all three cases, congestion and multiple thromboses being present in case 1, reticulo-endothelial proliferation and erythrophagocytosis being outstanding in case 2, and simple congestion of the pulp characterizing case 3.

The unusual activity of the hemolysins, which appear to be of endogenous origin and which possess a strong lytic action on cells of their own type as well as on those of group O, serves to set them apart from the known isohemolysins. As far as we are aware, no hemolysin has yet been reported which is active against group O cells.

The antilytic action of normal human blood serum may have some bearing on the therapeutic value of blood transfusions, which have usually been curative in acute hemolytic anemia. Because of the gradual diminution in activity of the hemolysins with continued recovery of the patients, it is possible that a causal relationship existed between the presence of the hemolysin and the development of the hemolytic syndrome. The presence of large numbers of spherocytes in case 3 with their subsequent diminution in number in association with diminution of the titer of the hemolysin makes us suspect that the spherocytes and the hemolytic factor were closely related.

Acute hemolytic anemia may be related to paroxysmal (cold) hemoglobinuria, paroxysmal nocturnal hemoglobinuria (Marchiafava), acquired hemolytic icterus, hemolytic splenomegaly (Banti) and even to congenital hemolytic jaundice.

Although in most cases acute hemolytic anemia appears to respond effectively to transfusions, the possibility must not be forgotten that in occasional cases the condition is so severe that the more radical measure of splenectomy must be employed.

DISCUSSION

FREDERIC PARKER JR.: Has Dr. Dameshek any knowledge of the character of this hemolysin? Is it absorbed by the red cells to some degree?

WILLIAM DAMESHEK: There are many questions to be answered with reference to this hemolysin. As yet we are not aware of its chemical nature, and we know little of its immunologic characteristics. It is absorbed to some extent by the red cells when they are left in contact with the serum. A hemolytic factor was present in various fractions of the splenic extract, whereas an extract of normal spleen was negative for hemolysin. This is a very unusual finding, and we hesitated to present it, because many cases of acute hemolytic anemia have been described since 1909 without the presence of a hemolysin being suspected except by Chauffard and Vincent (1909).

FREDERIC PARKER JR.: It is evident that the hemolysin is heat stabile, since addition of complement to heated serum restored the hemolytic activity.

MORTIMER WARREN: Does the hemolysin disappear if the spleen is removed?

WILLIAM DAMESHEK: The hemolysin gradually diminished after splenectomy in all three cases. At the end of four months, slight hemolytic activity was present in case 1.

AN INTERN: Did paroxysmal hemoglobinuria occur?

WILLIAM DAMESHEK: Hemoglobinuria occurred in one case. Hemoglobinuria and hemolytic jaundice are closely related. If hemolytic activity is great and rapid, hemoglobinuria will be found; if it is slight and prolonged, hemolytic anemia (icterus) will be seen without hemoglobinuria. Various gradations and combinations are possible, and many clinical syndromes illustrative of these gradations have been reported.

A PHYSICIAN: After transfusion in these cases, was there ever an increase in icterus?

WILLIAM DAMESHEK: A marked increase in icterus occurred after transfusion in cases 2 and 3 on two occasions.

A PHYSICIAN: Do you attribute the improvement to splenectomy or to transfusion?

WILLIAM DAMESHEK: In the first two cases several transfusions had done nothing to ameliorate the patients' symptoms or to improve the blood picture. In fact, the patients kept going consistently downhill. The dramatic response to splenectomy was extremely striking. The therapeutic effect of splenectomy took place almost under one's eyes, within a few hours; the red blood cell count then rose more than 200,000 a day. One must conclude that splenectomy was a life-saving measure in these cases.

CHRONIC OCCLUSION OF THE INFERIOR VENA CAVA (INCLUDING THE RENAL VEINS) AND OF THE PORTAL VEIN, PRESENTING THE CLINICAL PICTURE OF THE NEPHROTIC SYNDROME: REPORT OF A CASE. H. A. DEROW.

A survey of the literature fails to reveal a case of chronic thrombosis of the inferior vena cava, renal and portal veins presenting the clinical picture of the nephrotic syndrome.

A 15 year old American Jewish school boy began to suffer with attacks of nausea, vomiting and anorexia twenty-two months before admission, followed five months later by an attack of left costovertebral pain, which was diagnosed as pyelonephritis and nephrosis. One month later, pain developed in the right lower quadrant of the abdomen, radiating to the right groin and right costovertebral region and across to the midepigastrium, accompanied by spasm and tenderness in the right lower and upper abdominal quadrants. After arrangements had been made for exploratory operation, there developed edema and tenderness of the inner portion of the right thigh, followed by edema of both legs and of the scrotum and abdominal wall and tenderness and spasm over the left abdominal quadrant. The edema disappeared several weeks later. Nine months before admission, edema of the legs recurred, followed by swelling of the abdomen. Three months later, the patient presented thrombophlebitis of the right external jugular vein and questionable similar involvement of the right subclavian vein. Prominent veins appeared on the abdominal and lower thoracic wall. Three days before admission, he suffered from chills, fever, nausea and vomiting, followed by severe diarrhea and coma. Examination gave normal results except for distention of the abdomen with fluid, prominence of the superficial veins of the abdomen and lower part of the chest and slight pitting edema of the legs. The blood pressure was normal throughout the illness. Laboratory examinations revealed no anemia or impairment of renal function but showed massive albuminuria, a few red and white blood cells, casts and double refractile bodies in the urinary sediment, hypercholesteremia, and hypoproteinemia with reversal of the albumin-globulin ratio. Death ensued shortly after admission.

Postmortem examination revealed pneumococcic peritonitis, enlarged kidneys, old recanalized and fresh fibrous thrombi in the inferior vena cava and in the portal and tracheal veins, and old, recanalized thrombi in the renal veins. Microscopic examination of the kidneys revealed no abnormality of the glomerular epithelium, endothelium and basement membrane. The tubules showed changes which probably occcurred post mortem. Heart, lungs, liver, spleen and pancreas were without lesion on gross and microscopic examination.

DISCUSSION

J. B. HAZARD: How frequent is thrombosis of the portal vein?

HARRY A. DEROW: Fairly common.

FREDERIC PARKER JR.: What was the blood pressure in this case?

HARRY A. DEROW: The blood pressure was normal throughout the illness.

FREDERIC PARKER JR.: Have you any idea as to the etiology of this thrombosis?

HARRY A. DEROW: No.

SIDNEY FARBER: What was the relation of the thrombotic process to the renal symptoms?

HARRY A. DEROW: I do not believe that the nephrotic syndrome was due directly to the thrombotic process in the veins. It is possible that it was an associated picture. I have not seen any reference in the literature to this picture produced by venistasis or by the induction of thrombosis in the renal veins.

SIDNEY FARBER: Was this thrombosis complete?

HARRY A. DEROW: The thrombosis was complete. The thrombi all showed marked recanalization, and some of these channels showed fresh thrombi. This was particularly evident in the inferior vena cava and portal veins.

FRANCIS P. MCCARTHY: Was there any evidence of pulmonary infarction?

HARRY A. DEROW: No.

FREDERIC PARKER JR.: It is an interesting point that with complete occlusion of the renal veins there was no change in blood pressure and the nonprotein nitrogen remained normal throughout.

A STUDY OF OCCLUSIONS AND ANASTOMOSES OF THE CORONARY ARTERIES BY INJECTION PLUS DISSECTION. M. J. SCHLESINGER.

All pathologists have noted repeatedly the lack of correlation between the site of complete occlusion, either fresh or old, in a main branch of a coronary artery and any infarct, recent or healed, in a corresponding area of the myocardium. This inconsistency has been ascribed to the presence of an anastomotic or collateral circulation, although this is seldom demonstrated in a particular heart. These anastomoses are supposed to increase with age.

This confusing situation has been reinvestigated by a new technic which permitted detailed study of an x-ray picture of all the coronary arteries seen simultaneously in one plane. These arteries received an injection, at 150 mm. of pressure, at 45 C., of an x-ray opaque injection mass consisting of lead phosphate agar tinted a different color for each artery. A newly devised incision permitted unrolling the fresh, unfixed heart so that all the large coronary arteries were in one plane, with no confusing overlapping in possible anastomotic zones. All narrowed or occluded arterial branches were easily seen and studied. Dissection of the arteries receiving the injection demonstrated the path of distribution of the colored masses.

Thirty-five hearts from patients from 50 to 80 years of age were examined by this method. In twenty normal hearts with no occlusions of the coronary arteries there were no anastomoses. In fifteen hearts with occlusions of branches of the coronary arteries, rich anastomoses were found, forming a collateral circulation bridging these gaps, but none elsewhere. In five of the latter group there were healed, well vascularized infarcts. In one heart with a fresh infarct, definite but obviously inadequate anastomotic channels were seen leading to the infarcted area.

On the basis of these observations it was concluded that anastomoses between branches of the coronary arteries do not increase with the age of the patient unless coincidentally there are also slow occlusions of branches of the coronary arteries. Then and there functional anastomoses always develop and to a marked and usually adequate degree. Too rapid occlusion, before adequate anastomoses develop, leads to infarction.

DISCUSSION

KENNETH MALLORY: This shows how superficial the usual examination is. Has Dr. Schlesinger ever been able to make an injection into a blood vessel in an infarcted area? Is occlusion in the majority of cases due to thrombosis on top of atheroma or just to atheroma?

M. J. SCHLESINGER: One actually finds vessels large enough to receive an injection. Thrombosis, as a rule, precedes infarction. In slowly progressive occlusion, anemic infarcts do not form. By the time occlusion is complete, there is enough anastomosis to compensate for it.

B. E. CLARKE: Actually, are new channels formed or is there just dilatation of preexisting channels?

M. J. SCHLESINGER: There are channels which may open at high pressure. Using the same fixed technic of making injections into vessels in hearts with occlusion, one cannot demonstrate anastomotic vessels smaller than 40 microns. Whether they are smaller vessels that increase their diameter or new vessels it is hard to say. Probably, they are small vessels which increase in diameter.

FREDERIC PARKER JR.: When injections were made into these hearts, did the blood pressure correspond to the blood pressure during life?

M. J. SCHLESINGER: Yes. It was about 150 mm. of mercury.

The pressure during life brings up another point which I am just beginning to study. The pressure during life, which is normally 120, is not steadily present. The normal pressure may rise to a higher zone for a short time. My associates and I are just beginning to study the effects of a higher than normal pressure.

Books Received

THE HISTOPATHOLOGY OF MALARIA WITH SPECIAL REFERENCE TO THE FUNCTION AND ORIGIN OF THE MACROPHAGES IN DEFENCE. William H. Taliaferro, Professor of Parasitology, University of Chicago, and H. W. Mulligan, Malaria Survey of India, Indian Research Fund Association. Indian Medical Research Memoirs, no. 29. Paper. Price, 5 shillings. Pp. 138, with illustrations. Calcutta: Thacker, Spink & Co., 1937.

PRACTICAL NEUROANATOMY. Joseph H. Globus, Associate Professor of Neuroanatomy and Neuropathology, New York University and Bellevue Hospital Medical College; Associate Neuropathologist and Adjunct Neurologist, the Mount Sinai Hospital, New York. Price, $6. Pp. 350, with illustrations. Baltimore: William Wood & Company, 1937.

INVESTIGATIONS INTO THE NATURE AND CHARACTERISTIC FEATURES OF POST-NORMAL OCCLUSION. Matthew Young, Elsa Johnson, Corisande Smyth and Mildred Still. Medical Research Council, Special Report Series, no. 225. Paper. Price, 1 shilling, sixpence. Pp. 93. London: His Majesty's Stationery Office, 1937.

GENITAL ABNORMALITIES, HERMAPHRODITISM AND RELATED ADRENAL DISEASES. Hugh Hampton Young, M.A., M.D., Sc.D., F.R.C.S.I., D.S.M., Professor of Urology, Johns Hopkins University; Visiting Urologist, Brady Urological Institute, Johns Hopkins Hospital. Cloth. Price, $10. Pp. 649, with 534 illustrations. Baltimore: William Wood & Company, 1937.

THE COLLAPSE THERAPY OF PULMONARY TUBERCULOSIS. John Alexander, M.D., F.A.C.S., Professor of Surgery, University of Michigan; Surgeon-in-Charge, Division of Thoracic Surgery, Department of Surgery, University of Michigan Hospital. Cloth. Price, $15. Pp. 705, with 367 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1937.

MEDICAL USES OF RADIUM: SUMMARY OF REPORTS FROM RESEARCH CENTRES FOR 1936. Medical Research Council Special Report Series, no. 226. Paper. Price, 1 shilling. Pp. 41, with 6 illustrations. London: His Majesty's Stationery Office, 1937.